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MOVEMENTS OF THE TONGUE IN SWALLOWING

By J. WHILLIS, *Guy's Hospital Medical School*

The movements of swallowing have been described by a number of observers, notably Barclay (1930, 1936), and, more recently, Johnstone (1942), but observations by direct vision of the movements of the tongue have not, so far, been recorded.

This paper is a record and analysis of observations made by slow-motion cinematography on a patient in whom it was possible to see the tongue through a wide gap in the left cheek made in the course of operation for sarcoma (Fig. 1). The alveolar process of the left maxilla had been removed, and the cheek was missing from just behind the angle of the mouth back to the ramus of the jaw, and from the zygomatic arch above to the lower jaw below. The patient was edentulous. Though the conditions are admittedly abnormal, some useful information can be gained from the case.

Photographs were taken at 64 frames per second and projected at 16 frames per second. Some photographs were also taken at 16 frames per second, but the movements were so fast that analysis was impossible. Examination of the patient was made during the swallowing of fluid and of solid material.

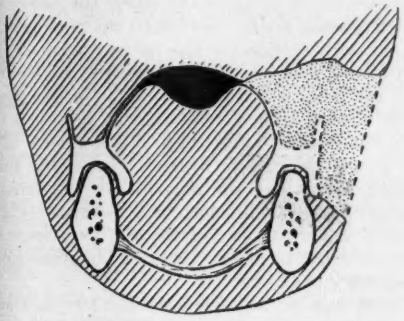

Fig. 1. Coronal section of the mouth showing the grooved upper surface of the tongue and the tubular space between it and the palate. The stippled area indicates the extent of the deficiency in the tissues.

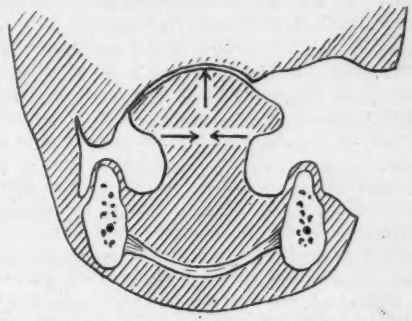

Fig. 2. Coronal section of the mouth showing the effect of contraction of the transverse fibres of the intrinsic musculature.

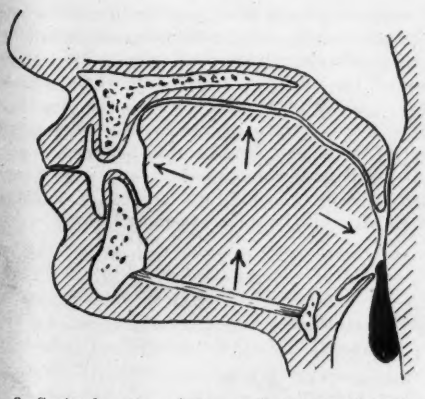

Fig. 3. Sagittal section of the mouth showing the effect of the mylohyoid in phase 2.

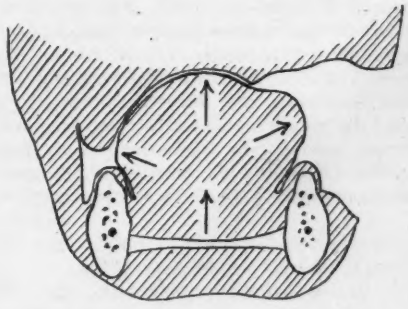

Fig. 4. Coronal section of the mouth showing the effect of contraction of the mylohyoid in the patient with the deficiency in the cheek.

OBSERVATIONS

The swallowing of fluids

Two phases were observed in the swallowing of fluids. In the first the tongue acted as a squirt transferring the fluid from the front to the back of the mouth. In the second phase the mylohyoid muscle came into action and forced the fluid from the oral into the laryngeal part of the pharynx.

Phase 1. The jaws are brought firmly together, the buccinator contracts and the tongue is fixed by the action of its extrinsic muscles so that its tip rests against the palate just behind the incisive papilla. At the beginning of this phase the upper surface of the tongue is grooved so as to form, with the palate, a tubular space in the upper part of the mouth (Fig. 1). The grooving is due to the contraction of the superior longitudinal and genioglossus muscles. Next, a groove (Fig. 2) appears on the side of the tongue, first at the tip and later extending progressively backwards, until it is apparent along the whole length of the anterior two-thirds. This is brought about by successive contraction of the lower transverse fibres of the intrinsic musculature and relaxation of the genioglossi, lengthening of the tongue being prevented by coincident contraction of the longitudinal intrinsic muscles. The effect of this grooving of the sides of the tongue is to bulge the dorsum upwards and so produce a progressive obliteration of the tubular space between the tongue and the palate (Fig. 2). In this way the fluid is squirted through the oropharyngeal isthmus into the oral part of the pharynx.

Phase 2. This follows phase 1 immediately and is associated with contraction of the mylohyoid muscle. The effect is shown in Figs. 3 and 4. This muscle, by raising the floor of the mouth, compresses the tongue so that it tends to bulge in any direction possible. In the normal mouth bulging laterally or forwards is prevented by the teeth, so that the posterior part of the tongue bulges backwards into the oral part of the pharynx, obliterating its lumen and driving its contents into the laryngeal part. In the present case the tongue is free to bulge laterally through the gap in the cheek as well as backwards into the pharynx (Fig. 4).

The swallowing of solids

In taking into the mouth solids which do not need to be bitten previously, the first movement of the tongue is, as it were, anticipatory. It is protruded, by contraction of the transverse intrinsic muscle, so that its tip lies in contact with the lower lip. The tongue is then retracted and carries the food backwards in the mouth where the movements of mastication finally carry it to the posterior part of that cavity. Since, in this patient, such mastication as was possible was done entirely on the right side, the part played by the tongue in this process could not be studied. It was seen, however, that, as in the case of fluids, there was a contraction of the mylohyoid muscle which squeezed the tongue against the roof of the mouth and completed the passage of the bolus into the pharynx (Fig. 3). Finally, saliva and food debris were evacuated from the mouth by the process described in phase 1 of the swallowing of fluids.

There was no leakage from the left side of the mouth, the tongue forming an effective seal with the palate and keeping the food entirely on the right side.

SUMMARY

An analysis is made of the movements of the tongue in swallowing by slow-motion cinematography.

In swallowing fluids, two phases are seen. In phase 1 the intrinsic musculature of the tongue is used to squirt the fluid through the mouth. In phase 2 contraction of the mylohyoid bulges the base of the tongue into the oral pharynx.

In swallowing solids phase 2 only is used except when the mouth is being cleared of saliva and debris after the bolus has been swallowed.

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MOTOR LOCALIZATION AND THE EFFECTS OF NERVE INJURY ON THE VENTRAL HORN CELLS OF THE SPINAL CORD

By G. J. ROMANES (*Beit Memorial Fellow*), *Anatomy School, Cambridge*

INTRODUCTION

The regenerative processes which follow injury to a peripheral nerve have been studied extensively, but little is yet known of the significance of the changes which take place in the cell-bodies of the injured nerves.

An investigation has been made of the changes in the ventral horn cells of the spinal cord in new-born rodents to determine whether any correlation between cell morphology and nerve regeneration could be established. Although nerve regeneration is said to be more satisfactory in young mammals than in old (Ranson, 1906; Elkington, 1944) it has long been known that the retrograde changes in injured nerve cells are much more rapid and extensive in the former than in the latter (Vulpian, 1868; Gudden, 1872; Ranson, 1904, 1906). An attempt to clarify this apparent contradiction is made in the present work.

MATERIAL AND METHODS

120 new-born mice were used together with a few new-born rats and rabbits. All operations were performed under ether anaesthesia and had to be carried out quickly on account of the rapidly rising death rate under prolonged anaesthesia. After being allowed to survive for periods ranging from 1–133 days the animals were killed with chloroform. Controls were obtained from litters of known age and from some animals in which operation had failed to injure a nerve. The spinal cords of the latter were just the same as those of the normal controls.

Four types of operation were performed:

1. Amputation of individual segments of a limb to determine whether each was represented in a single motor cell column of the spinal cord.

2. Removal of single muscles or groups of muscles in a segment of the limb to see if either is supplied by a compact nucleus within a motor cell column of the spinal cord.

3. Crushing of the sciatic nerve without any muscle removal to determine whether the presence of denervated muscle at the periphery alters the fate of the injured cells.

4. Crushing of a purely sensory nerve to eliminate the possibility that the motor-cell degeneration is a transneuronal and not a retrograde phenomenon in the new-born.

(1) *Amputation of varying amounts of one hind-limb*

These operations were carried out by crushing the whole limb at the desired level with a fine clamp which was held in position for about 1 min., cutting off the distal part of the limb and sealing the adherent skin edges with collodion. Such operations were carried out through ankle, knee or hip. In the case of the hip it was seldom possible to remove all the thigh muscle and it was found impracticable to carry out a more prolonged dissection in view of a rapidly rising mortality rate with prolonged anaesthesia. These animals were killed from 1 to 130 days after operation.

(2) *Removal of the various groups of muscles which lie in the leg*

The muscles were exposed through an incision along the lateral aspect of the leg and the tendons picked up with a fine hook which was drawn up to the knee thus stripping the muscles from their attachments. The tendons were then cut and the muscles removed. This method gave minimal haemorrhage and allowed an accurate removal of individual muscles or groups of muscles without injury to the adjacent tissues even in new-born mice. On account of the small size and non-medullated nature of the nerves in new-born rats and mice it proved impossible to be certain that nerves lying close to the muscles had not been damaged, but every animal was carefully examined to find out whether such an injury had occurred to the main nerves.

Two main groups of muscles were removed:

(*a*) The muscles supplied by the lateral popliteal nerve, excluding the peronei in a few cases.

(*b*) The triceps surae. Here only the lower two-thirds of the gastrocnemius was removed to avoid injury to the popliteal nerves. These animals were all killed 7 days after operation.

The wound was dressed as before without suture, the skin edges being apposed.

(3) *Crushing of the sciatic nerve*

This operation was performed on a series of new-born animals which were killed at intervals up to 133 days. Owing to the difficulty of isolating the non-medullated nerve and the need for speed, a blind technique was used. A small incision was

made in the skin on the lateral aspect of the leg just caudal to the greater trochanter. Through this a fine sharp hook was passed deep to the thin layer of biceps femoris, a small piece of which was picked up together with the subjacent sciatic nerve and the whole crushed with a fine clamp.

(4) *Crushing of the saphenous nerve*

In a small series the saphenous nerve was crushed on the medial aspect of the knee by the same method.

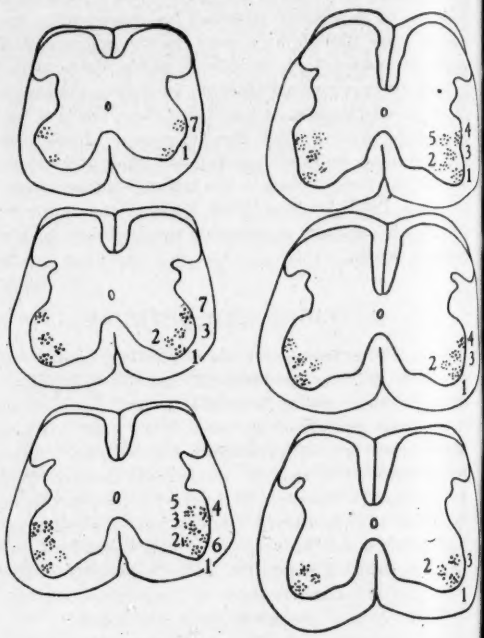
When the animals were killed the lumbo-sacral region of the spinal cord was removed together with the vertebral column and fixed in Jenkin's mixture after removal of excess muscle. The tissues were embedded in paraffin and serial transverse sections at 15–25 μ were cut. In the older mice the spinal cord was removed after fixation to facilitate embedding and thus avoid the undue brittleness which is a feature of the mouse spinal cord. In a few cases the vertebral column and spinal cord were fixed and impregnated by de Castro's method and serially sectioned at 15 μ. The sections were stained with toluidine blue and from them reconstructions of the motor-cell groupings on both sides of the lumbar enlargement were made by Elliott's (1942) method. In addition one spinal cord was reconstructed with wax plates into which a lead shot was placed corresponding to each ventral horn cell. The whole model consisting of a series of transverse sections was fused together and stereoscopic X-ray photographs taken to aid in interpreting the groupings. Counts of the number of ventral horn cells of the typical motor type were also made on both sides of the spinal cords, and graphs of their distribution were produced by adding the number of cells in each group of five sections. These counts were all started at the caudal end of column 7 which marks the inferior extremity of the lumbar enlargement and only those cells lying in the lateral part of the ventral horn and showing distinct nucleoli were counted.

In some of the experiments involving crushing of the sciatic nerve the whole limb was removed, treated by de Castro's silver impregnation method and serially sectioned at 15 μ to show the extent of nerve regeneration and to determine the degree of injury to the nerve. In others pieces of the nerve, several millimetres proximal to the site of crushing, were removed together with the corresponding part of the normal sciatic nerve. They were stained partly by de Castro's method to show the axis cylinders and partly with 1% osmic acid to show the myelin sheath in the older animals. Transverse sections were cut at 5 μ in paraffin and counts were made by projecting the sections through a microscope on to squared paper at a magnification of $\times$ 650.

OBSERVATIONS

(1) *The normal anatomy of the motor cell groupings in the lumbar enlargement of the mouse*

Seven separate longitudinally arranged cell columns are present in the lateral part of the ventral horn in the control spinal cords and on the normal sides of the operated spinal cords. These are numbered in order of appearance on following the sections cephalo-caudally. In the mouse the motor-cell groupings are rather indistinct but by the use of the various reconstruction methods it was possible in a large number of cases to determine their arrangement accurately. The arrangement of these columns as seen in transverse section is shown in Text-fig. 1.



Text-fig. 1. Series of diagrams to show the ventral horn cell groupings at different levels of the spinal cord.

Column 1 appears in the upper lumbar cord situated in the ventro-lateral margin of the ventral horn and is continued almost to the caudal extremity of the enlargement in this situation. In its most caudal part its cells are much smaller than elsewhere but still form an easily recognizable group with well formed Nissl granules.

Column 2 appears slightly caudal to column 1 and lies on its dorso-medial aspect passing down into the sacral spinal cord to disappear just cranial to column 1.

Column 3 begins in the upper lumbar region dorsal to column 1. Further caudally it sweeps in a dorso-medial direction to lie dorsal to column 2 and medial to column 4. It continues caudally to a point between the termination of columns 1 and 2 and here lies ventral to column 7.

Column 4 appears in the mid-lumbar region dorso-lateral to column 3. It passes caudally to end in the sacral region leaving a distinct gap between itself and column 7.

Column 5 begins in the lower half of the lumbar spinal cord just dorsal to column 3 and dorso-medial to column 4. Throughout its length it is closely related to column 3 and is fused with it in many places. It ends at the same level as column 4.

Column 6 is a small dorso-lateral extension of column 1 from which it is barely separated. It lies in the lower lumbar and upper sacral segments.

Column 7 arises just caudal to column 4 and at first lies dorsal to column 3. It extends further caudally than any other column of the lumbar enlargement, its caudal extremity marking the lowest extent of the lateral group of motor cell columns.

Table 2 shows the number of cells in the caudal 40 sections of column 7 on the normal and operated sides at intervals after amputation. In the knee amputations column 5 sometimes appeared to be reduced in size but this never amounted to a disappearance and in many cases it was quite normal. For this reason column 5 has been represented by a ± in the knee amputations. It is found that following injury to the sciatic nerve high in the thigh column 5 was never altered though all muscles below the knee were denervated. Thus column 5 does not innervate muscles below the knee.

From the above facts it follows that, if the cell loss is the result of sectioning the axons of the ventral horn cells, then the foot is represented in column 7, the leg in column 4 with perhaps some cells in column 5 and the thigh in columns 3 and 5.

Three graphs of the numerical distribution of ventral horn cells on both sides of the lumbo-sacral spinal cord following amputation through the left ankle, knee and hip respectively are shown in Text-fig. 2 (1–3). In these and subsequent graphs the caudal part of the lumbar enlargement is on the left of the graph while the continuous and dotted lines represent the number of ventral horn cells on the right and left sides of the spinal cords. Abscissae: number of sets of five sections. Ordinates: number of cells. The distance between the continuous and dotted lines is the number of missing ventral horn cells. Beneath each graph are diagrams of corresponding regions of the spinal cord in transverse section. It is clear from these graphs that the cell loss spreads up the spinal cord with each increase in the amount amputated, corresponding to expectation in view of the distribution of the spinal nerves.

Table 1. *Amputation at birth*

Types of amputation	No. of animals	Numerical loss of cells: Average	Max.	Min.	Columns: 1	2	3	4	5	6	7
Foot	12	282	318	254	+	+	+	+	+	+	−
Knee	16	638	729	585	+	+	+	−	±	+	−
Hip	6	850	1008	760	+	+	−	−	−	+	−

(2) *Effects of amputation and muscle removal*

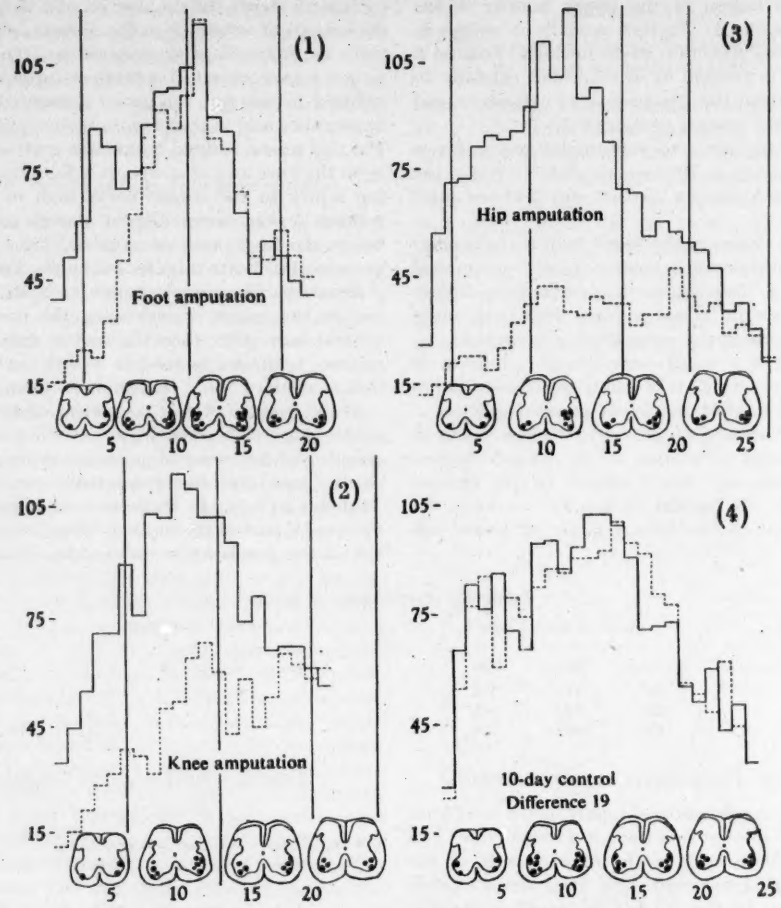
Following amputation of a part of the hind limb in new-born mice or rats there is a considerable loss of ventral horn cells in the affected side of the lumbo-sacral spinal cord. This loss, which reaches its maximum by the 5th to 7th day after amputation, is numerically the same for similar operations and a standard columnar loss occurs with each amputation.

Table 1 gives the results from a series of amputations.

Since it was impossible to remove all the thigh musculature by the method used the average number of cells lost fell well below the probable maximum but the greatest loss shown in Table 1 was from an animal where all but a fragment of thigh muscle was removed. In regard to the columns, a + represents no change while a − indicates virtual absence of a column. Unfortunately, the proximity of the cell groupings to each other makes it impossible to count them individually except in the case of column 7 which is isolated in the lowest part of the enlargement. In the latter column the cell destruction rose to 90–98 % of its total complement at the end of one week in every operation.

Table 2. *Number of cells (column 7)*

Days after operation	No. of cells: Normal side	Operated side	% difference
1	328	317	− 3·3
2	340	257	− 24·4
3	262	210	− 19·8
4	300	114	− 62·0
5	252	59	− 76·5
7	257	28	− 89·0
8	318	13	− 96·0
12	257	27	− 90·0
16	275	10	− 96·3
32	227	7	− 98·0



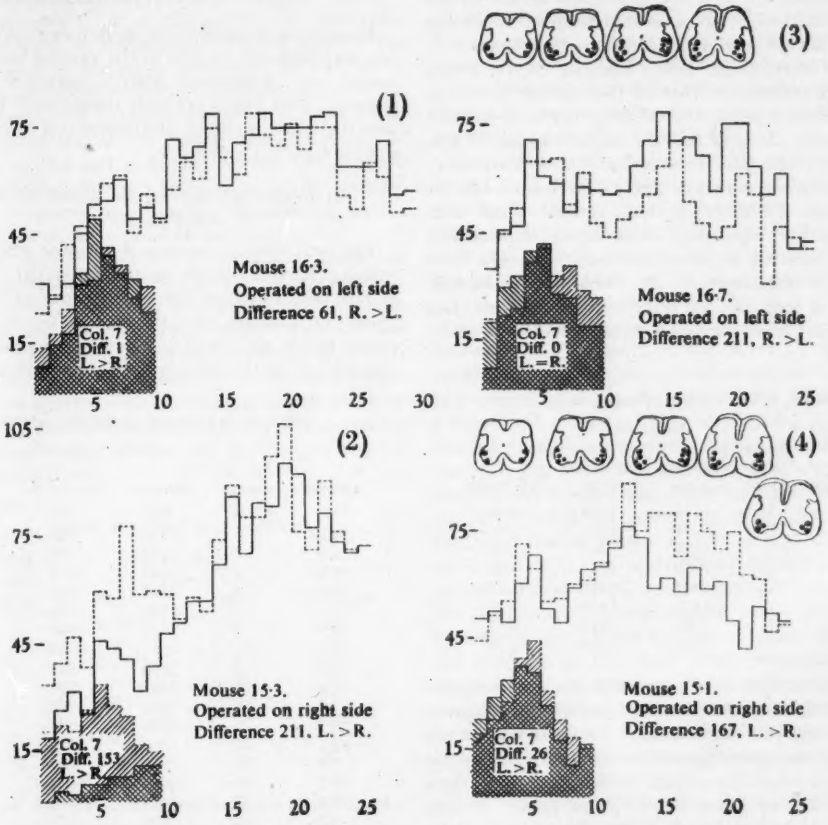
Text-fig. 2. Graphs of the number and distribution of ventral horn cells on both sides of the lumbo-sacral enlargement of the spinal cord 10 days after amputation of parts of the left hind limb at birth, with a control for comparison. Abscissae: number of groups of five sections. Ordinates: number of cells.

Table 3. *Muscle removal*

Animal	Age at death (days)	No. of cells: Normal side	No. of cells: Operated side	Difference	Columns affected	Column 7: Normal side	Column 7: Operated side	Difference	Muscles removed
16·5	7	1506	1336	170	4	238	237	1	Anterior tibial group and peronei
16·7	7	1472	1261	211	4	284	284	0	" "
16·8	7	1518	1327	191	4	203	206	3	" "
15·5	7	1563	1427	136	4 (7)	207	180	27	Gastrocnemius and soleus
15·1	7	1567	1400	167	4 (7)	240	214	26	" "
16·1	7	1812	1761	51	—	240	234	6	Tibialis anterior
16·3	7	1752	1691	61	—	250	251	1	"
15·3	7	1655	1444	211	7	202	49	153	Less complete removal of gastrocnemius and soleus. Posterior tibial nerve cut in mid-leg
16·4	7	1588	1124	464	7, 4	228	20	208	Removal of gastrocnemius and soleus. Posterior tibial nerve cut in mid-leg

In order to place a further check on these results muscle groups were removed from the leg as described above and the results are shown in Table 3. The figures given in brackets indicate a small but visible loss of cells; where no figures are given the loss was too small to be localized anatomically.

It is obvious that where extensive damage to the posterior tibial nerve has been avoided, excision of the leg muscles, whether flexors or extensors, leads to degeneration in column 4 alone. This is graphically shown in Text-fig. 3 which gives the numerical distribution of the ventral horn cells of both sides throughout the lumbar enlargement in four cases of muscle removal at birth. The small cross-hatched graphs show the distribution of cells in column 7 only. The serial numbers of the mice on the graphs correspond with those given in Table 3 from which the extent of the operation may be determined. It can be seen also that when gastrocnemius and soleus are removed there is always a greater loss of cells in column 7 than after removal of the anterior tibial group of muscles. Whether this means that a few cells in column 7 supply the plantar flexor muscles of the ankle or that there is always some trauma to the posterior tibial nerve is not known, though the extreme degree of degeneration of column 7 with foot amputation alone makes the latter more likely. When the posterior tibial nerve is destroyed the loss to column 7 is considerable and the degeneration in column 4 varies with the extent of the muscle removal. These experiments reinforce the evidence that column 7 supplies the foot and column 4 the leg.



Text-fig. 3. Graphs of the number and distribution of ventral horn cells on both sides of the lumbo-sacral enlargement of the spinal cord after muscle removal. Inset graph is of column 7. For nature of operation see Table 3.

A further check consisted in showing that if the effects of three partial operations (i.e. (1) removal of the foot; (2) excision of the leg muscles supplied by

the lateral popliteal nerve; (3) excision of gastrocnemius and soleus) are summated (Fig. 6(6)) the result obtained is very similar to the effect of knee amputation but with more cells of column 4 persisting in the middle of the enlargement, presumably representing the deep flexor muscles of the leg.

The possibility still remained that the cellular degeneration was not due to section of the motor axons but rather to a destruction of incoming sensory nerves such as may occur after dorsal root section (Warrington, 1898; Barron, 1933). However, it is not known whether the ventral horn cells are influenced when the sensory nerve is injured peripherally. To test this the saphenous nerve was crushed at the knee in a series of new-born mice. The results are shown in Table 4, which reveals no significant difference in the ventral horn cells following this procedure, so that the amputation and muscle removal experiments must cause a true retrograde degeneration. In Table 4 the absolute number of cells counted is different in each case but all include the sciatic and femoral nerve segments of the cord.

Table 4. *Crushing of saphenous nerve*

Animal	Age at death (days)	No. of cells: Normal side	No. of cells: Operated side	Difference
1	7	1774	1808	+34
2	7	1541	1520	−21
3	7	1871	1902	+31
4	7	1580	1608	+28
5	7	1864	1849	−15
6	7	1424	1473	+49
7	7	1681	1688	+ 7
8	7	1732	1755	+23

New-born mice were used for all the above experiments because in the few new-born rabbits operated on the only cell degeneration obtained was in the column corresponding with column 7 in the mouse no matter what the extent of the operation. Rats were found to react similarly to mice. It was thought probable that the difference in the results between mice and rats on the one hand and rabbits on the other depended upon the fact that the rabbit is more mature at birth. This is partly borne out by the first of a series of experiments performed on a new-born litter of mice in which amputations through the knee were carried out at daily intervals after birth and each mouse killed 6 days after operation. Table 5 gives the results of counts of column 7 on the two sides. Though incomplete this series suggests that there is an increasing resistance of the cells which appears in the first week of extrauterine life when the cells undergo considerable histological changes and medullation occurs in the spinal cord and peripheral nerves.

Table 5. *Amputation at knee*

Age at operation (days)	Age at death (days)	No. of cells: Normal side	No. of cells: Operated side	Difference	% difference
1	7	255	129	126	56
2	8	239	103	136	57
3	9	217	172	45	21
5	11	275	184	91	33
6	12	197	163	34	17

In operations carried out on new-born mice there is a standard loss of cells in the ventral horn of the spinal cord associated with a standard muscle removal. This loss is not only numerically the same but the topographical localization of the missing cells is readily predictable.

(3) *Results of crushing the sciatic nerve in new-born mice*

The next problem was to determine whether the cellular changes already seen in the spinal cord were in any way modified by the presence at the periphery of muscles denervated by injury to the sciatic nerve, and if the degeneration persisted what effect it had on the regeneration of the injured nerve.

Table 6. *Crushing of sciatic nerve*

Animal no.	Age at death (days)	No. of cells: Normal side	No. of cells: Operated side	Difference
4·1	2	1702	1624	78
1·1	3	1723	1243	480
9·3	4	1892	1295	597
9·4	6	1547	681	866
12·1	7	1706	909	797
9·6	8	1192	580	612
12·2	11	1687	968	719
2·1	12	1576	952	624
12·3	15	1458	898	560
2·2	16	1475	800	675
12·4	22	1662	837	825
12·5	25	1567	713	854
2·3	32	1636	838	798
12·7	39	1696	981	715
7·2	65	1612	884	728
2·5	83	1683	991	692
4·5	97	1528	839	689
7·3	104	1419	746	673
8·2	133	1589	904	685

Table 6, which is compiled from part of the series, shows the number of cells lost at different ages after operation. It shows that, following crushing of the sciatic nerve, the number of motor type cells in the ventral horn on the affected side is grossly reduced and that this reduction reaches its maximum by the 6th day. The counts given include many cells whose axons have not been injured as both sciatic and femoral nerve segments are counted and many cells in the former belong to trunk, rump and thigh muscles which have not been denervated.

It can be seen that the number of cells lost varies considerably but lies mostly between that found on knee and hip amputation (Table 1). Also, in considering the older animals, it is clear that there is no marked tendency for the missing cells to be replaced, so that the damage inflicted in the first 6 days shows no sign of being made good in the ensuing 133 days. The graphic representation of this series is shown in Text-figs. 4 and 5.

Unfortunately, it is impossible to determine whether there is a significant loss of cells in the ventral horn of the unoperated side. This is due to the difficulty in determining the exact extent of the spinal cord which has been counted in a series where the length of the cord is rapidly increasing and the relation of the cell groupings to the spinal roots (the intervals between which are shown by vertical lines in some of the graphs) appears to be inconstant from one animal to another.

Table 7 and Text-fig. 6 (1–5), show a few counts of ventral horn cells from control spinal cords for comparison with Table 6 and Text-figs. 4 and 5. These show that similar numbers of ventral horn cells are found in the control spinal cords and on the normal side of the operated animals, the counts having been taken over approximately the same length of spinal cord. They also demonstrate that the cell counts on the left and right side in the normal present no significant difference.

Table 7. *Control animals*

Age of control (days)	No. of cells		Difference
	Right	Left	
2	1920	1923	3
3	1545	1551	6
4	1540	1529	11
6	1502	1466	36
8	1404	1416	12
12	1385	1377	8
39	1683	1694	11
104	1536	1520	16

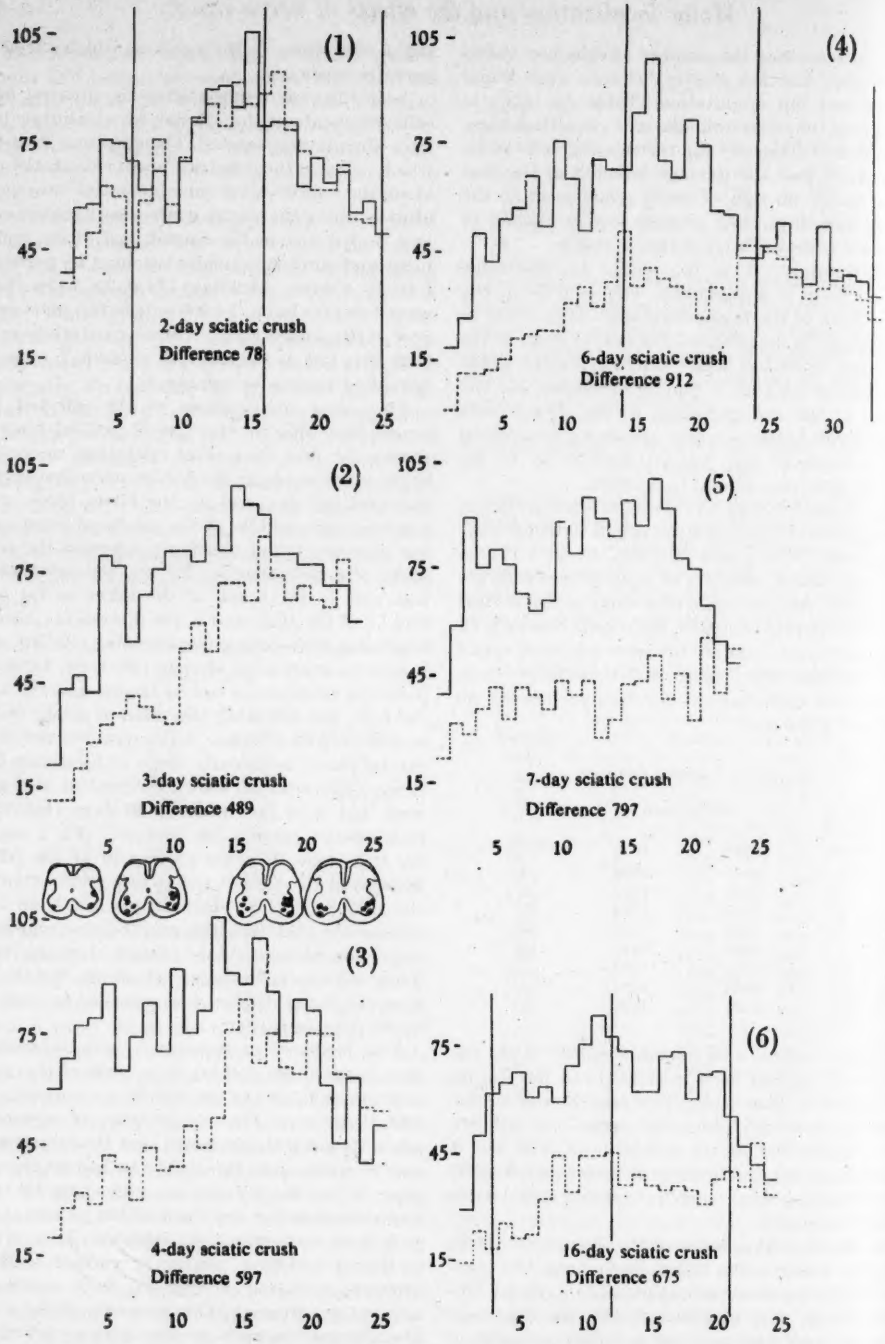
In both control and operated spinal cords the number of ventral horn cells tends to fall during the first week after birth. This may be due to the increasing ease with which the motor-type cells are differentiated from other ventral horn cells, but it may be a natural phenomenon as there is histological evidence of degenerating cells in control spinal cords during this period.

The anatomical position of the degenerated cells following sciatic nerve injury lends some confirmation to the localization experiments. All the reconstructions give identical results showing that columns 7 and 4 are lost and a variable number of cells missing from the caudal part of column 3. The latter loss appears to be proportional to the amount of denervation of the hamstrings which varies with slight alterations in the point at which the sciatic nerve is crushed.

As in the case of amputation the greatest loss of cells occurs about the 4th day by which time many have already degenerated. The graphs in Text-fig. 4 which depict the ventral horn cell distribution along the lumbo-sacral enlargement at various ages after crushing the sciatic nerve show that degeneration begins first in the caudal part of the enlargement and spreads cranially reaching its permanent form by 6 days. At 2 days (Text-fig. 4 (1)) there is some loss of cells on the left side in the most caudal part of the enlargement. Histological study reveals that this loss is confined to column 7 only later spreading to involve column 4.

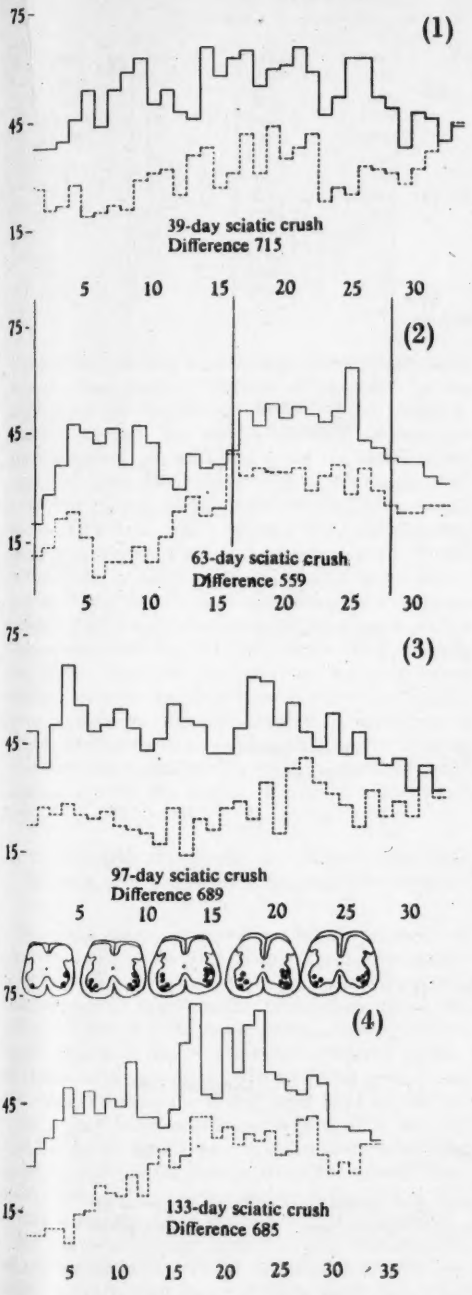
The silver preparations of the affected limbs reveal that, despite the loss of ventral horn cells during the first week after operation, nerve fibres begin to grow along the sciatic nerve between the 2nd and 3rd day and by the latter fibres can be traced to the middle of the popliteal fossa and in one case are found ramifying amongst the muscle fibres of gastrocnemius. By the 4th day fibres are traceable in the trunk of the nerve as far as the middle of the tibia and a few motor-end plates are beginning to form in gastrocnemius. At the end of 7 days these are more obvious (Pl. 1, fig. 14) and the posterior tibial nerve can be traced into the sole of the foot, but following this there is a long delay in innervating its muscles. A few motor-nerve endings can be found in an early stage of formation in the flexor digitorum brevis at the end of the second week but it is not until 30–40 days that typical motor-nerve endings are present. (Pl. 1, fig. 16). By this stage they are present in all the affected muscles of the leg but not to any great extent, and the number at 133 days seems less than in the normal leg. At first the motor-nerve endings are more complicated than normal, forming bizarre loops and knobs on the muscle fibres, but the adult type (Pl. 1, fig. 13) has been reached in most cases by 60 days or earlier.

The recovery of function proceeds steadily so that between 60 and 70 days most of the animals are using all parts of their limb in a normal manner and there is no obvious evidence of abnormality when they run about except that the limb is smaller and in some cases the ability to spread the toes is poor. When the animals are picked up by the tail and their hind legs kept just off the ground the limb is held in a characteristic position. It is extended at the hip and knee, plantar flexed at the ankle and strongly adducted so that the foot comes to lie across the perineum. This seems to be the result of the greater strength of the muscles which have retained their nerve supply intact and to the relatively greater strength of the plantar flexors which are the first to be re-innervated. This posture



Text-fig. 4.

Text-figs. 4, 5. Graphs of the number and distribution of ventral horn cells in the lumbo-sacral enlargement of the spinal cord at varying intervals after crushing the left sciatic nerve.



Text-fig. 5.

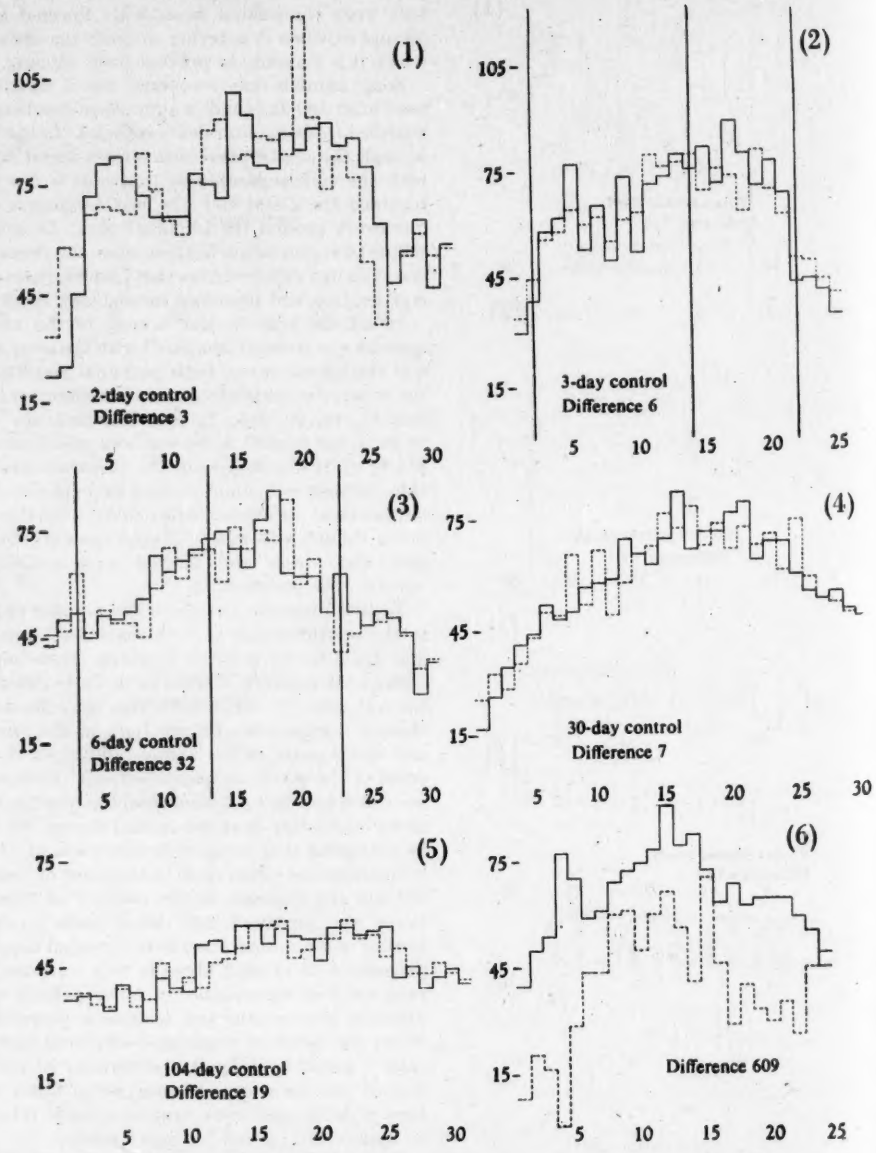
is found up to 100 days or longer, becoming progressively less obvious. The last muscles to function are the interossei as is seen by the spreading of the toes when the animal is suddenly lowered to the ground or when it is trying to grasp the surface on which it is standing to prevent itself slipping.

Some animals only recovered use of their interossei after 100 days and in a few cases function only returned to the plantar flexor muscles. In the latter animals the sciatic nerve was always found divided with the ends separated so that only a few fibres regained the distal end, the rest forming a dense meshwork around the proximal part. Despite the failure of regeneration in these cases the spinal cord was in no way different from that found in cases where regeneration had produced satisfactory results.

In all the animals the volume of the affected muscles was reduced compared with the normal side and the sciatic nerve, both proximal and distal to the crush, was markedly reduced in diameter (Pl. 1, figs. 17, 18, 20, 21). In the older animals, up to 80 days, the normal nerve was well medullated and pearly white in colour while the damaged nerve was thin, reddish white and showed no evidence of the normal cross striations. After 80 days the damaged nerve though still much thinner than its counterpart was whiter and showed some evidence of normal cross striation.

Table 8 gives the counts of the number of fibres in the sciatic nerves of both sides, both proximal and distal to the point of crushing. Those marked with an asterisk were stained by de Castro's method, the rest with 1 % osmic acid. This table shows that there is a gross loss of fibres both in the proximal and distal parts of the nerves, and when the two parts of the nerve are compared it is obvious that the distal part has not been completely refilled with fibres originating from the central stump. So it can be presumed that no considerable amount of axon bifurcation has taken place at the point of crushing. Though the decrease in the number of fibres between the proximal and distal parts is slightly greater in the normal than in the crushed nerve, the percentage of missing fibres is very constant. This suggests that the number of fibres passing out in branches between the two sections is proportional. When the point of crushing is observed histologically it would be difficult to determine its presence were it not for many escaping nerve fibres which form a dense meshwork around what is otherwise an apparently normal but small nerve.

Table 8 also shows that there is no significant increase in the number of nerve fibres in either the proximal or distal parts of the nerve up to 133 days. This agrees with the cell counts in the ventral horn of the spinal cord and reinforces the evidence that the limb must be supplied by the few remaining nerve cells.



Text-fig. 6 (1–5) Graphs of the number and distribution of ventral horn cells in the lumbo-sacral enlargement of the spinal cord of control animals at various ages. (6) A composite graph reconstructed from three spinal cords.

Table 8. *Crushing of sciatic nerve*

Mouse no.	Age (days)	No. of fibres in sciatic nerve proximal to crush: Normal side	Operated side	Difference (%)	No. of missing ventral horn cells	No. of fibres in sciatic nerve distal to crush: Normal side	Operated side	Difference (%)
12·1*	7	3024	1094	63	797	2880	973	66
12·2*	11	3478	1357	61	722	3221	1308	59
12·3*	15	4370	2459	43	560	—	—	—
12·3	15	2758	1105	60	560	—	—	—
12·4	22	3083	946	69	825	2870	897	68
12·6	31	3320	1617	51	620	3006	1514	50
12·7	39	4496	1688	62	715	3978	1516	61
7·2	65	5197	2615	50	728	—	—	—
2·5	83	4290	2133	50	692	3814	1905	50
4·5	97	3848	1669	57	689	3532	1536	56
8·2	133	3912	1332	66	685	3101	1201	63

* De Castro stained.

It seemed possible that sensory nerve fibres might produce motor-nerve endings in muscle. In six adult mice the saphenous nerve was cut and implanted through the biceps into the denervated gastrocnemius. In all these cases the nerve fibres grew out from the central end of the saphenous nerve but merely ramified longitudinally amongst the muscle fibres, never forming the typical transverse arrangements of the terminal part of the motor nerve or anything resembling a motor-nerve ending. These results were not extended as a more detailed paper by Gutmann (1945) appeared giving very similar results. It is obvious from the small size of the posterior root ganglia and the great number of fibres missing from the crushed sciatic nerve compared with the number of degenerated ventral horn cells, that cells of the sensory system have suffered a similar fate and in numbers commensurate with the excess of sensory over motor fibres.

(4) *Histological changes in the ventral horn cells following any peripheral nerve injury in new-born mice*

From the above observations on the sequence of disappearance of ventral horn cells in the spinal cord following injury it is obvious that some cells must disappear more rapidly than others and so no ordered series of changes can be expected by studying these cells at different ages after injury. In fact all stages of degeneration can be found in any one age studied during the first 7 days after operation. Even 1 day after operation several cells can be found in an advanced state of degeneration, but the majority show little change from the normal side apart from having a rather scanty cytoplasm which is finely granular when stained with toluidine blue.

By the second day more obvious changes are apparent; the nuclei of cells on the operated side are more excentric and in some cases bulge out from the cytoplasm, which may lie wholly on one side of the nucleus (Pl. 1, fig. 9). In other respects the cells do not show any gross change though the cytoplasm is scanty and shrunken around the nucleus. In a few cases the cells are swollen and a clear space can be seen surrounding them which is due to excessive shrinkage in treating the tissue (Pl. 1, fig. 11). The cytoplasm is finely granular and stains less intensely than the aggregations of Nissl substance seen in the unaltered cells. Some cells show an enlarged nucleolus which is irregular in outline, but stains densely with toluidine blue (Pl. 1, fig. 1).

In the third day many of the cells have taken on a spherical shape and several have no recognizable dendrites. Two main types of these cells are seen:

(1) A type of cell closely resembling the classical picture of chromatolysis with a small, grossly excentric nucleus pressed up against the cell boundary. The cytoplasm is pale and swollen with a few aggregates of Nissl substance at the periphery and especially close to the nucleus (Pl. 1, fig. 10).

(2) A more common type of cell which shows a reddish blue reticular cytoplasm staining darkly and not swollen. In the later stages of degeneration the cytoplasmic reticulum appears to coalesce into a series of minute globules which stain less darkly. In this type of cell the nucleus is central, or nearly so, and contains several intranuclear bodies (Pl. 1, figs. 2, 3, 6, 7), most of which are characterized by having a size similar to that of the original nucleolus but stain light blue and have a definite internal structure. This takes the form of radially arranged strands of darkly staining material joining a small central mass to a peripheral rim of similar substance (Pl. 1, fig. 6). In more advanced stages of degeneration these intranuclear bodies have the appearance of a sphere of tightly packed granules and later fuse together into a homogeneous mass (Pl. 1, figs. 4, 8 A). Throughout this process of degeneration the cytoplasm and nucleus gradually shrink and finally the nuclear outline disappears leaving the dark intranuclear bodies apparently free in the centre of a

mass of cytoplasmic granules (Pl. 1, figs. 4, 8 B). From this stage the cell either gradually shrinks, the cytoplasmic granules coalescing into a structureless mass which stains with a gradually increasing intensity until the nuclear remains are no longer visible and the cell is represented by a structureless globule of deep blue material lying in the space originally occupied by the cell (Pl. 1, fig. 5); or the cell boundary breaks down and cytoplasmic granules diffuse out into the surrounding tissues leaving only fragmentary remains of the cell.

The first type of cell (Pl. 1, fig. 10) seems to undergo a similar series of changes with intranuclear alterations akin to those described above (Pl. 1, figs. 10–12). The cytoplasm also becomes granular and finally a homogeneous mass, but the cells tend to remain more voluminous throughout though the end point of degeneration seems to be indistinguishable from that in the other type of cell. This type of cell is much less common than that described above and is seen only occasionally and most often between the third and fourth days after operation, especially amputation. A third type of cell is occasionally found which shows an indefinite ragged outline. The nucleus is central and normal. No later stages which could be connected with this type were seen but it may give rise to the second type described above.

In all cases one or more neuroglial cells are found closely applied to the degenerating cells and as the number of cells gradually decreases it becomes clear that there is a higher concentration of neuroglial cells in the region of the degenerated cells than elsewhere.

Apart from the degeneration involving the ventral horn cells of the affected side very many globules similar to those described above are to be found throughout the spinal cord, particularly in the dorsal horns, though in smaller numbers on the unoperated side. On the normal side a few degenerating ventral horn cells are found. These are much less numerous than on the operated side but are more frequently found than in the spinal cords of control animals where an occasional ventral horn cell can be found in a degenerating state especially during the first 3 days after birth. Thus though some ventral horn cells degenerate normally in the new-born mouse the operations appear to affect those of the normal side to a slight extent. From the numerical results given previously it is obvious that there is no great loss of ventral horn cells on the normal side of the spinal cord following contralateral operations. It is of interest, however, to note that though the degenerating cells are uniformly scattered throughout the cell columns of the ventral horn in control spinal cords, they are most common in column 7 of the normal side of operated spinal cords.

Only a small fraction of the ventral horn cells shows these definite changes at any one time, so, unless the degeneration proceeds with extreme rapidity, it is difficult to see how it can account for the large number of cells lost in the short space of time occupied by the degeneration. On the other hand, there is no evidence that the cells undergo a process of dedifferentiation thus rendering them unrecognizable for, once a cell column has degenerated, the space which it leaves is virtually devoid of nerve cells except for the scattered few which fail to degenerate. It is presumed then that the degenerative phase of a single cell is extremely short. This is confirmed by the presence of cells in the last stages of degeneration one day after operation and by the sudden loss of cells occurring between the third and fourth days.

In order to strengthen the evidence for the degeneration already produced a series of spinal cords were stained by de Castro's method at intervals after the sciatic nerve had been crushed. This technique also failed to reveal any sign of the missing cells or their processes. Pl. 1, fig. 19 shows a section through the lower part of the lumbo-sacral enlargement of one of these mice 5 days after operation. On the operated side it is clear that not only is column 7 missing but the fibres which arise from its cells and form a conspicuous bundle passing to the ventral root on the normal side are also absent.

DISCUSSION

Following Nissl's (1892) description of the retrograde changes in nerve cells after injury to their axons, it has been implicitly assumed in the majority of text-books that a classical reaction of this type inevitably follows peripheral nerve trauma. That this is not the case in anterior horn cells of the spinal cord in many adult mammals was recognized by van Gehuchten (1900), Marinesco (1898), Bucy (1928), Geist (1933), Romanes (1941) and many others. In spite of this chromatolysis is frequently used as an experimental method for determining localization in the spinal cord. As a consequence of the variability of this reaction in motor cells of the spinal cord it is hardly surprising that as much confusion exists about this subject as 50 years ago.

Gudden (1872) is reputed to be the first to have demonstrated that in young animals nerve cells undergo a much more rapid and complete degeneration after injury. However, this was already known to Vulpian (1868) and has since been confirmed by many authors including Hayem (1873), Mayser (1877), Homen (1890), Pellizzi (1892), Ranson (1906), d'Abundo (1914), and Brodal (1940). Spinal localization does not seem to have been studied in

terms of this extensive degeneration which characterizes young animals.

The results of this study confirm the previous tentative suggestion concerning spinal localization (Romanes, 1941) and extend it by showing that a single segment of the limb, in this case the thigh, can be supplied by more than one column of cells. That this division is not necessarily due to a functional difference in the muscles is clear when it is seen that the flexors, evertors and extensors of the foot are represented in column 4 without any special localization for each group. It cannot be affirmed that every motor cell supplying a particular segment of the limb lies in a single cell group, but it is certain that the majority of them do so. Baumann & Landauer (1943) have shown that polydactyly in the fowl is associated with an increased number of cells in those two columns which correspond anatomically with the groups supplying the foot and the leg.

The failure of a significant number of ventral horn cells to degenerate following section of a sensory nerve virtually rules out the possibility that the cell changes described are of a transneuronal type, which previous experiments tended to suggest (Warrington, 1898, 1899; Tower, Bodian & Howe, 1941), although arterial or venous damage may have played an important part (Tower, 1937) as was demonstrated by Tureen (1936). The failure of cells to degenerate does not preclude the possibility that changes in the ventral horn cells of the spinal cord are enhanced by the coincident sensory degeneration.

On the other hand, the number of ventral horn cells which undergo degeneration on the normal side appears to be greater than in controls. This would suggest:

(1) That there is a transneuronal degeneration which may be of two types.

(*a*) Degeneration of motor ventral horn cells as a result of the destruction of cells conveying stimuli to them. It is interesting to note that Warrington (1899) obtained bilateral (mainly homolateral) degeneration of the ventral horn cells following unilateral posterior rhizotomy associated with spinal transection. This degeneration was always more intense than after either of these two operations alone.

(*b*) Degeneration of cells histologically indistinguishable from motor ventral horn cells but associated with ascending sensory systems. Such a system of cells has been suggested by Cooper & Sherrington (1940) whose evidence, though very suggestive, is by no means conclusive in the present state of our knowledge of the factors involved in retrograde degeneration or the possible division of labour amongst ventral horn cells. However, similar topographical results have been obtained by Tower *et al.* (1941), and Hare & Hinsey (1940). Despite the operations on the saphenous nerve the latter is still possible as Cooper & Sherrington consider that the 'spinal border cells' give rise to cerebello-petal fibres in the tract of Gowers and their degeneration might be maximal after section of proprioceptive rather than exteroceptive nerve fibres.

(2) That some of the ventral horn cells send their axons into the ventral root of the opposite side, a point on which this paper sheds no light.

With regard to the rate of degeneration of ventral horn cells, the present results agree fairly closely with those given by Brodal (1940) for the inferior olivary nucleus, but in addition they show that there is a spatial order of degeneration. This is seen in the greater rapidity with which cells supplying the foot degenerate as compared with those innervating the more proximal parts of the limb. This seems to be of some significance when combined with the observations that, in the new-born rabbit, only that cell column which supplies the foot degenerates whatever the extent of the amputation, while the remaining cells undergo minor histological changes involving a lysis of the Nissl granules. Thus it seems that, with increasing degrees of development, those cells which are embryologically first in the field (that is most ventrally situated in the ventral horns) are the first to become resistant to axonal section as well as the first to medullate their axons.

In contrast to Brodal's (1940) results on the inferior olive the preliminary investigation reported here on the age by which resistance to axonal section is developed in the ventral horn cells of the lumbar enlargement points to the development of such a resistance in many of the cells by the end of the first week after birth. That this resistance is always lower in the column supplying the most distal segment of the limb is obvious from the researches of Hayem (1873), Dreschfeld (1880), Pellizzi (1892), Warrington (1898), and Romanes (1941) who found this column most severely affected by peripheral nerve injuries in adult animals. This fact may have some bearing on the failure to obtain good results with nerve regeneration in the most peripheral parts of the limbs. In this series of experiments it is clear that the ventral horn cells degenerate after severance of their axons, irrespective of the presence or absence of the peripheral muscles, and that the injury has not merely resulted in a failure of the cells to continue their development, as seems to be the case when limb rudiments are removed prior to innervation (Hamburger & Keefe, 1944).

Thus there are three stages in the life of a motor neurone:

(1) An undifferentiated stage which requires

attachment to a muscle for its further differentiation.

(2) A stage of growth and further differentiation of the cell during which it responds to axonal damage by rapid degeneration.

(3) A stage, epitomized by the adult state, during which the cell resists trauma to its axon and separation from its muscle at least for a very considerable period during which it may undergo gradual atrophy unless reconnected with a muscle mass. Such atrophy has long been recognized from the study of old amputation cases (Romanes, 1942, bibliography).

The ventral horn cells thus differ from the dorsal root ganglion cells which show the classical form of chromatolysis in all cases of peripheral nerve injury in rodents at any age after birth. However, it is clear from this study that these cells likewise undergo a rapid degeneration after section of their peripheral processes in new-born rodents and in foetal rats (Hall & Schneiderhan, 1945). These authors consider that the deficit of cells found on the operated side is an aplastic phenomenon as they saw no degenerative phases (cf. Ranson, 1909, in young animals); but the degeneration seen here is complete in less than 7 days and the earliest study carried out by Hall & Schneiderhan was between 4 and 5 days after an operation which corresponded closely to the present series of amputations, with the exception that foetal stages were used in which the rate of degeneration may be even more rapid than in the new-born.

It is not clear why the ventral horn cells should develop a relatively greater resistance to section of their peripheral processes than the posterior root ganglion cells. However, as the injured processes of these two types of cell are arranged in physiologically opposite directions, it is probable that during development the former cells come more and more under the influence of the descending tracts and spinal internuncial neurones for which they form a final common path, while the latter group retain their single relation with specific sensory end organs. It is known that section of the central processes from the dorsal root ganglia causes no change in their cells (van Gehuchten, 1900; Anderson, 1902; Hinsey, Krupp & Lhamon, 1934; Tower, 1937; Hare & Hinsey, 1940; Lassek & Perry, 1944) though it may cause degenerative changes in ventral horn cells (Warrington, 1898, 1899; Barron, 1933) which may be similar aetiologically to those found in isolated spinal cords (Tower *et al.* 1941) after pyramidal section (Barron, 1933) or cortical damage (d'Abundo, 1914).

These results confirm Ranson's (1906) observation that there is no attempt on the part of the spinal cord to replace the degenerated ventral horn cells, so that although there are still many apparently undifferentiated cells in the spinal cord they are either not stimulated, or are incapable of being stimulated to further differentiation by the presence of denervated muscles. If Barron's (1943) supposition is correct this may be due to the fact that the remaining ventral horn cells have already an established dendritic field, or that the cells, though histologically undifferentiated, are no longer plastic.

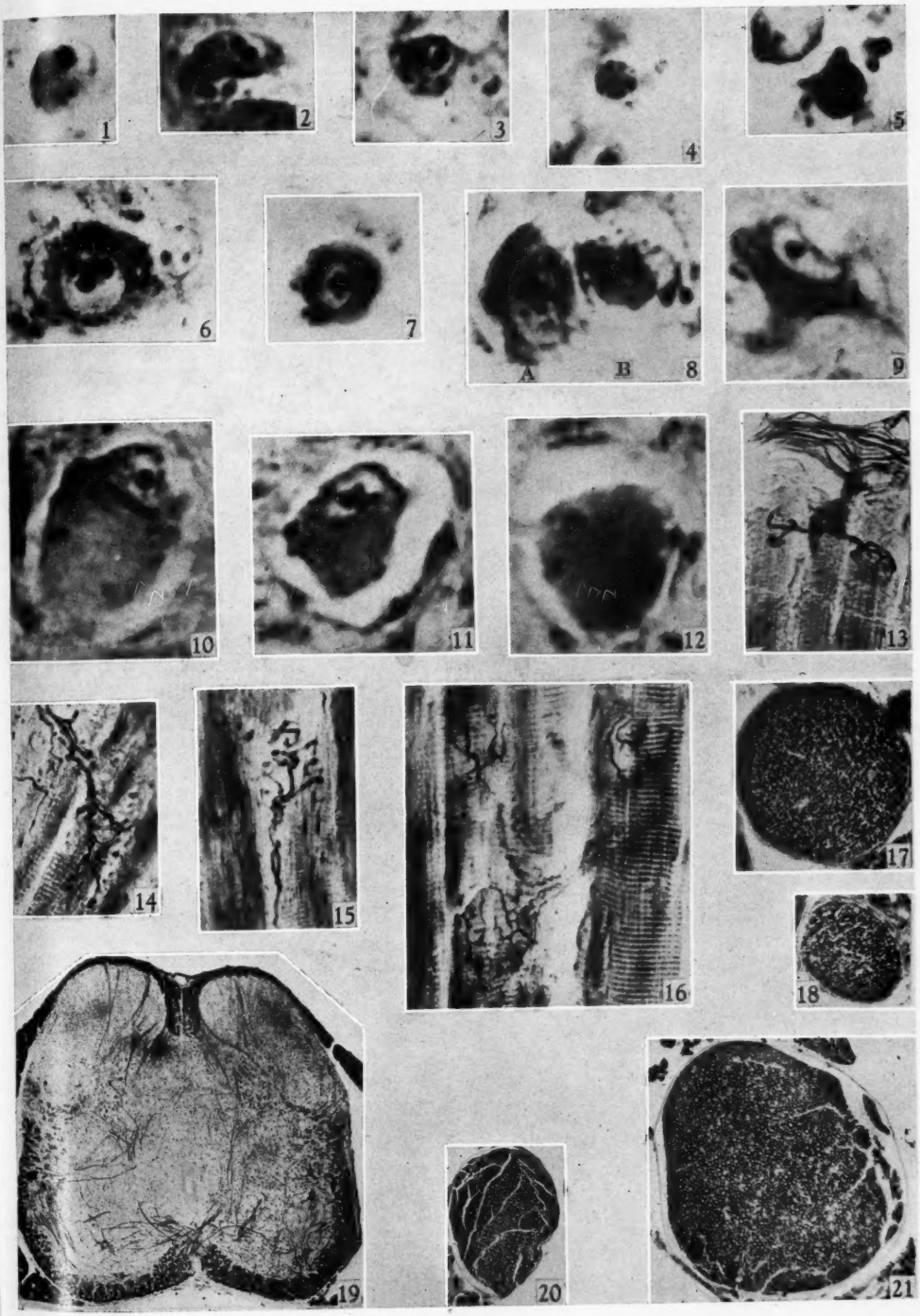
The most striking feature of the experiments is the degree of functional recovery occurring in animals which have lost 40–50 % of all the ventral horn cells of the limb region and a much higher percentage of the cells whose axons have been interrupted. This raises several questions as to the mechanism involved. The most important is to determine the origin of the regenerating fibres. The evidence points to the fact that they arise near the cut end of the nerve and presumably from the cells which have survived the trauma. This is shown by the rapidity with which regenerating sprouts appear and the absence of any cellular proliferation or differentiation in the spinal cord, which makes it most unlikely that the fibres have regenerated from the spinal cord.

The small number of motor cells, belonging to the sciatic nerve, which survive the injury is capable of re-innervating the denervated limb and yielding a functional result. Is each of the remaining cells connected with the same muscle or group of muscles as it was before the operation? In view of the type of operation used, the failure of the sciatic nerve fibres to show any significant degree of bifurcation at the point of injury and the fact that, despite many aberrant fibres passing out from it, the internal structure of the nerve shows little alteration, it would seem reasonable to presume that the relation between the cell groupings in the spinal cord and the peripheral segments of the limb has not been unduly disturbed.

Thus, although only a few ventral horn cells remain, co-ordinated function has returned to the denervated limb. It may be that the position in the spinal cord of a cell which innervates a denervated muscle is of much greater importance to the recovery of function than the density of innervation; the former maintains the spatial organization of the spinal cord which is probably of paramount importance for the maintenance of local reflexes and the correct action of descending tracts on a spinal cord of high organization and poor adaptability (Sperry, 1940 et seq.).

SUMMARY

1. Motor localization in terms of anatomical cell groups in the ventral horn of the lumbo-sacral enlargement in the spinal cord of young mice has been determined using Gudden's method.



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2. The stages in degeneration of the ventral horn cells of the spinal cord have been studied following amputation of various parts of one hind-limb or crushing of the sciatic nerve in new-born mice. Maximal degeneration of the cells occurs about the 4th day after operation and is complete by 7 days. A spatial order of degeneration has also been noted.

3. Despite the loss of ventral horn cells following injury to the sciatic nerve, regeneration occurs with recovery of function between 60 and 100 days after operation. There is no increase in the number of ventral horn cells up to this period.

4. Injury to the saphenous nerve at birth is unassociated with any anterior horn cell changes.

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EXPLANATION OF PLATE

Figs. 1–12. Anterior horn cells showing various stages of degeneration. ×1300.

Fig. 13. Normal mouse motor-nerve ending. ×560.

Fig. 14. Reforming motor nerve ending in gastrocnemius of a mouse 7 days after crushing sciatic nerve. ×560.

Fig. 15. Reforming motor-nerve ending in tibialis posterior of a mouse 32 days after crushing sciatic nerve. ×560.

Fig. 16. Three motor-nerve endings in extensor digitorum brevis of a mouse 44 days after crushing the sciatic nerve. ×560.

Figs. 17, 18. Transverse section of the right (17) and left (18) sciatic nerves taken at a point 4 mm. proximal to the level of crushing of the latter from a mouse 39 days after operation. ×126. Stain, de Castro.

Fig. 19. Transverse section through the lower part of the lumbo-sacral enlargement of the spinal cord of a mouse 5 days after crushing of the left sciatic nerve. ×70. Stain, de Castro.

Figs. 20, 21. Transverse sections of the left (20) and right (21) sciatic nerves at a point 4 mm. proximal to the level of crushing of the former. From a mouse 50 days after operation. ×70. Stain, osmic acid.

SOME EFFECTS OF PITUITARY ADRENOTROPIC HORMONE (PATH), EXTRACT OF SUPRARENAL CORTEX, AND COLCHICINE ON THE HAEMOPOIETIC SYSTEM

By J. M. YOFFEY and J. S. BAXTER, *From the Department of Anatomy, The University, Bristol**

Only scant information is available concerning the mechanism whereby the level of the blood cells is controlled, and a balance maintained between new cells entering the blood and old cells leaving it, or being destroyed while in the circulation. The lymphocytes would appear to lend themselves best to the investigation of such a mechanism, for we can in their case obtain an approximate idea as to both the numbers entering and the numbers leaving the blood.

The view that the blood cells are subject to hormonal rather than direct nervous control has long been favoured by many haematologists, partly because of the poor innervation of haemopoietic tissues, and partly because both in the blood and the parent tissues the cells are subject to continual displacement. In the case of lymphocytes and lymphoid tissue, a number of observations have in the past seemed to point to the suprarenals as an essential part of the control mechanism (Drinker & Yoffey, 1940). Recently, attention has been focused still more closely on the suprarenals since the discovery and investigation of pituitary adrenotropic hormone, or for short PATH.

On the question of terminology, a number of workers designate the adrenotropic hormone of the pituitary as adrenocorticotropic hormone, or briefly ACTH. This appears to be a somewhat loose designation, for other hormones are known to have an action on the cortex. Thus Vogt (1944) has shown that adrenaline stimulates cortical secretion. Further, the thyroid gland also has important effects on the suprarenal cortex (Hoskins, 1910; Ingle & Higgins, 1938; Swann, 1940). It may be that in many animals this is effected through the pituitary, but certainly not in all. In the pigeon, Miller & Riddle (1939) have shown that thyroxine induces cortical hypertrophy even in the hypophysectomized animal. Unpublished results by one of us (J.S.B.) indicate further that the action of the pituitary adrenotropic hormone, while it may be primarily on the cortex of the suprarenal, produces changes in the medulla also. For this reason the term pituitary adrenotropic hormone (PATH), indicating the specific origin of the hormone in question, as well as its possible action on the suprarenal as a whole, seems preferable to ACTH, and is accordingly employed in this paper.

Dougherty & White (1944) found that 'Single injections of pituitary adrenotropic hormone in mice, rats and rabbits produce within a few hours an absolute lymphopenia...'. White & Dougherty (1945) further noted that daily injections of PATH, continued for 15 days, produced in the blood of mice (*a*) increase in haemoglobin and red cells, (*b*) an absolute lymphopenia, and (*c*) an increase in polymorphs; concurrently there was diminution in weight of lymph nodes and thymus (Dougherty & White, 1943). They also reported unpublished studies which indicated 'that in mice injected with adrenotropic hormone daily for a period as long as one year, a persistent absolute lymphopenia is present'. Simpson, Li, Reinhardt & Evans (1943) reported atrophic changes in lymph nodes and thymus after administration of PATH, and stated that in the lymph nodes a 'flushing out' of lymphocytes occurred. Ingle (1938) noted atrophy of the thymus following the administration of cortical extract, as also did Crede & Moon (1940) after PATH. Wells & Kendall (1940) described thymic involution after cortico-sterone. Reinhardt & Li (1945) observed a fall in the lymphocyte content of thoracic duct lymph in rats after PATH, and a similar observation has been made by Yoffey, Reiss & Baxter (1946) in cats.

Dougherty & White (1945) studied in detail the early histological changes in mouse and rabbit lymphoid tissues after the administration of PATH, and cortical extract.

The present experiments were undertaken to confirm the findings of White & Dougherty (1945) and to see whether the administration of PATH and extract of suprarenal cortex for a period of 1 month, as compared with 15 days in their published work, would give more clear-cut results.

MATERIAL AND TECHNIQUE

The experiments were performed on twenty-six healthy Wistar rats and two rabbits. Cortrophin (Organon)† was the preparation of PATH used, standardized in Sudanophobic units as described by Reiss, Balint, Oestreicher & Aronson (1936). The extract of suprarenal cortex employed in the rats was Eschatin,‡ standardized in dog units (Harrop, Pfiffner, Weinstein & Swingle, 1932); in the rabbits another commercial extract, also

* Aided by a grant from the Colston Research Fund.

† We are indebted to the courtesy of Organon Laboratories Ltd., for supplies of Cortrophin.

‡ Kindly provided by Messrs Parke, Davis and Co.

standardized in dog units, was employed. Rat blood was obtained from the tail vein, rabbit blood from the ear. Before incising a tail vein, rats were anaesthetized with ether, a procedure which Crafts (1944) and others have shown to be without significant effect on the blood count. For the routine staining of blood films Wright's stain was employed, and in the differential counts not less than 200 cells were counted. Reticulocyte staining was done with a dried film of brilliant cresyl blue, counting 1000 cells.

Sections of lymph nodes—mesenteric or cervical—and of thymus were cut at 6μ after fixation in Zenker-formol and paraffin embedding, and were stained with iron haematoxylin and Dominici's eosin-orange G-toluidin blue stain.

The experiments fall into four groups:

I. Twelve rats which were given daily intraperitoneal injections of PATH, cortical extract, or both (for dose see Table 1) for periods ranging from 3 to 21 days. In addition these rats were given between 0·1 and 0·2 mg. colchicine per 100 g. body weight 16–17 hr. before being killed. Three blood counts were made on each animal: count A immediately before beginning the injection of PATH or cortical extract, count B 3–5 hr. after the first injection, and count C just before the animal was killed. These experiments lasted for periods ranging from 3 to 21 days.

II. Eight rats were given daily intraperitoneal injections of PATH or cortical extract (for dose see Table 2) for 27–28 days, but no colchicine. Colchicine had been given to group I in order to study more accurately details of lymphocyte mitoses. At that time we were not aware that colchicine itself had a markedly destructive effect on lymphoid tissue in rats, exactly like that noted in mice by Lits, Kirschbaum & Strong (1938). Accordingly in the animals of group II colchicine was omitted, but the administration of PATH and cortical extract continued for 4 weeks. Two blood counts were made in this group: count A, immediately before beginning the injections, and count B before killing the animals.

III. Six colchicine controls. In three animals colchicine was injected and the animals were killed 16–17 hr. later, for histological examination of lymph nodes and thymus. In another three animals the blood picture in response to colchicine was also investigated. Count A was made, then colchicine was injected (0·15 mg./100 g. body weight), and 16 hr. later count B was made and the animal was killed.

IV. Two rabbits, in which the red and white blood cells were counted over a period, to get the range of normal fluctuations, and then a cortical extract (*c.* 12·5 dog units per kg. body weight) was given subcutaneously every day for 16 and 40 days respectively.

RESULTS

Blood. Table 1 gives the results of the first series of twelve rats. Three blood counts were made in each experiment. Count A is the normal count before the injection commenced, count B was taken at times varying from 3 to 5¼ hr. after the first injection, and count C after the injections had continued for times ranging from 3 to 21 days. In this group count C was complicated by the administration of colchicine about 16 hr. before it was made, but count B shows the uncomplicated action on the blood lymphocytes of PATH and cortical extract, either separately or in combination. In eight out of the twelve experiments there was a marked fall in the blood lymphocytes; in one there was an appreciable rise, and in two no significant change; in one experiment count B was not recorded. On the whole the short term effect of PATH and of cortical extract in rats seems to be a fall in the blood lymphocytes, as noted by Dougherty & White (1944).

Table 2 presents the results of the second series of eight long-term rat experiments in which PATH and cortical extract were given over a 4-week period. PATH alone has induced a rise in the blood lymphocytes, but cortical extract alone or together with PATH a fall. In the case of the red cells also (Table 2) the results suggest the possibility that the action of PATH may differ from that of cortical extract, though the number of experiments is not big enough to draw definite conclusions.

In the twelve experiments quoted in Table 1, the long-term results are difficult to assess, for they are in part due to the colchicine. Eight experiments showed a fall in the blood lymphocytes, four a rise. In the case of the red cells four showed a fall and eight a rise, and in some the rise suggests an erythraemia. Furthermore, immature red cells (normoblasts) and white cells (a few myeloblasts, but chiefly myelocytes and metamyelocytes) appeared in the blood, sometimes in considerable numbers. At the same time marked basophilia was noted, though reticulocytes were not counted in this group. The appearance of immature red and white cells in rat's blood following the administration of colchicine, as well as a marked polymorphonuclear leucocytosis, agree with the findings of Dixon & Malden (1908), and later of the Brussels School (for further details see Lits *et al.* 1938).

The result in one rabbit experiment is presented in Text-fig. 1, which shows the daily blood lymphocyte counts during an experiment lasting 16 days, preceded by a control period of 22 days. The blood lymphocytes show a marked drop from the third to the eighth day of the experiment and then rise again. But the drop is of the same order as had occurred previously during the control period, and in any case was only temporary. In the second experiment, where injections were given

Table 1. *Blood lymphocytes per cu.mm.* (*ordinary type*) *and red blood cells in* 1000*'s per cu.mm.* (*black type*) *after daily intraperitoneal injection into rats of Cortrophin* (C), *Eschatin* (E) *or Cortrophin + Eschatin* (C + E)

Count A immediately before injections commenced.
Count B 2½–5½ hr. after first injection.
Count C* after repeated injections for 3–21 days.

No. of animal	Count A	Count B	Count C	Time between A and B (hr.)	Time between B and C (days)	Colchicine mg./100 g. body weight	Substance used and daily dose per 100 g. body weight
29	19,920	25,036	14,425	5	8	0·15	C—0·14 S.U.
	—	—	—				
30	19,190	11,270	7,035	5¼	7	0·2	E—0·2 D.U.
	8,720	**8,320**	**6,800**				
31	12,670	13,254	7,870	4½	7	0·2	E—0·19 D.U.
	7,580	**6,960**	**6,900**				
32	16,826	7,090	10,937	4¼	17	0·175	C + E—0·2 S.U. + 0·2 D.U.
	7,760	**7,020**	**7,740**				
33	13,532	—	8,487	—	18	0·2	C + E—0·4 S.U. + 0·2 D.U.
	7,160	clotting	**12,720**				
34	14,998	11,240	13,920	4	18	0·18	C—0·4 S.U.
	8,620	**7,680**	**7,760**				
35	5,022	5,010	9,835	4¼	20	0·2	E—0·5 D.U.
	6,300	**5,920**	**8,380**				
36	13,993	8,597	17,036	4	4	0·2	C—0·25 S.U.
	8,420	**7,810**	**9,780**				
37	11,652	5,938	8,042	3	21	0·15	C—0·11 S.U.
	6,830	**7,260**	**9,340**				
38	8,050	3,310	5,040	5¼	21	0·1	C—0·22 S.U.
	7,020	**7,780**	**9,740**				
39	14,283	7,333	27,277	2¾	21	0·1	E—0·4 D.U.
	7,440	**7,640**	**5,260**				
40	9,045	4,673	15,379	3¾	3	0·1	C—0·67 S.U.
	7,960	**7,300**	**9,860**				

* Count C in this group of experiments was complicated by the injection of colchicine 16–17 hr. before the count was made. See text.

C = Cortrophin, dosage in Sudanophobic units (S.U.). E = Eschatin, dosage in dog units (D.U.).

Table 2. *Blood lymphocytes per cu.mm.* (*ordinary type*), *red blood cells in* 1000*'s per cu.mm.* (*black type*) *and reticulocytes after daily subcutaneous injection into rats of Cortrophin* (C), *Eschatin* (E) *or Cortrophin + Eschatin* (C + E)

Count A immediately before injections commenced.
Count B 27–28 days after first injection.

No. of animal	Count A	Count B	Reticulocytes %		Time between count A and B (days)	Substance used and daily dose per 100 g. body weight*	Weight in g.	
			Count A	Count B			Initial	Terminal
44	6,703	9,854	0·0	3·2	28	C—3·0 S.U.	333	292
	7,460	**7,500**						
45	6,612	8,595	0·4	6·4	27	C—1·5 S.U.	329	292
	8,120	**6,840**						
46	6,974	10,999	1·2	4·8	28	C—4·5 S.U.	324	350
	8,840	**7,720**						
48	15,742	9,397	1·6	3·8	28	C + E—1·5 S.U. 1·0 D.U.	239	246
	10,060	**8,800**						
51	16,700	12,822	1·8	3·1	27	C + E—3·0 S.U. 1·0 D.U.	238	257
	7,900	**9,004**						
54	12,205	9,507	2·2	5·2	27	E—0·5 D.U.	230	256
	7,600	**8,120**						
55	15,506	9,800	2·5	4·2	27	E—1·0 D.U.	245	250
	8,140	**8,040**						
56	13,117	9,330	3·5	5·9	27	E—2·0 D.U.	251	287
	8,320	**7,960**						

* For explanation of terms see Table 1.

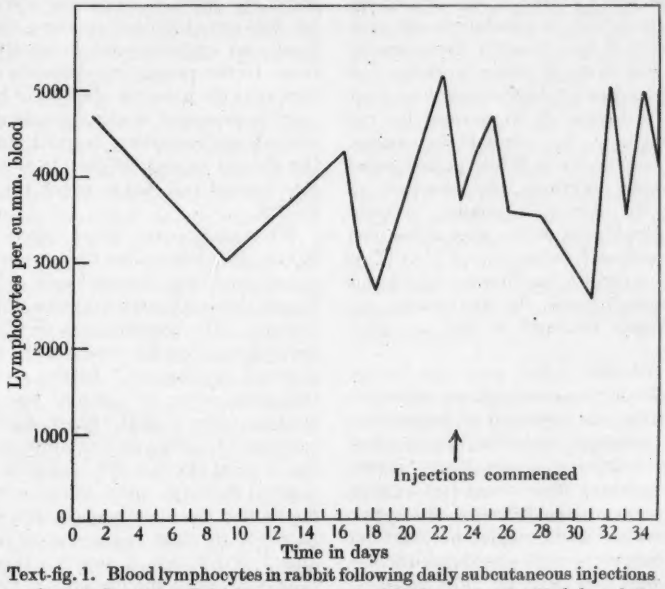
daily from 16 April to 26 May 1940, the blood lymphocytes were: 1 May, 3860 per cu.mm.; 20 May, 3180 per cu.mm.; and 27 May, 3620 per cu.mm. Again these counts were of the same order as counts performed during a preceding control period. It will be seen, then, that continued administration of cortical extract in the two rabbits had no marked effect on the lymphocytes in the peripheral blood.

HISTOLOGICAL OBSERVATIONS

Colchicine. As far as concerns the action upon lymphoid tissue of PATH and cortical extract, the experiments in group I were invalidated by the use of colchicine, which has a profoundly destructive effect on lymphoid tissue. Figs. 1 and 2 (Pl. 1) are low and high power views of a normal rat lymph node, figs. 3 and 4 corresponding views of a lymph node from an animal treated with colchicine. Fig. 4 shows the widespread pyknosis and karyorrhexis to which colchicine gives rise.

PATH. Figs. 5 and 6 (Pl. 2) illustrate the effect of the prolonged (4 weeks) administration of PATH on lymph nodes. The effect is quite definite if one compares these with the corresponding figures (Pl. 1, figs. 1 and 2) of the normal lymph node. The zone of cortical lymphoid tissue is greatly diminished in depth, while the medullary sinuses are dilated, giving rise to the appearance of 'flushing-out' noted by Simpson *et al.* (1943). But the lessened amount of lymphoid tissue which is present seems quite normal, both in the cortex and the medulla, and mitoses could be observed in the germ centres (Pl. 2, fig. 6), though they did not appear to be very numerous.

Cortical extract. An unexpected result was obtained with cortical extract. As may be seen in figs. 7 and 8 (Pl. 2), the lymphoid tissue of the nodes is not only not atrophied, but on the contrary appears to be more active than normal. The germ centres show numerous mitoses, and the cells are closely packed. The diffuse lymphoid tissue surrounding the germ centres is dense, and extends into the greater part of the medulla. The reaction, in fact, is a slight but definite hyperplasia. The presence of numerous macrophages is also evident even in a low power view (Pl. 2, fig. 7), both in the germinal centres and in the diffuse lymphoid tissue. They stand out as lightly-staining areas and give rise to the appearance of 'pitting' described by Dougherty & White (1945).



Text-fig. 1. Blood lymphocytes in rabbit following daily subcutaneous injections of cortical extract. Initial control period of 22 days. On the 23rd day, daily subcutaneous injections commenced of aqueous cortical extract, 12·5 dog units per kg. body weight.

PATH + cortical extract. In two experiments in which PATH and cortical extract were given in combination (Pl. 3, figs. 9 and 10) the stimulating effect of the latter seems to have considerably neutralized the depressant effect of the former, as may be seen by comparing figs. 9 and 10 (PATH + cortical extract) with figs. 5 and 6 (PATH alone).

DISCUSSION

The differing reaction of lymph nodes to PATH and cortical extract

As has already been noted, the difference between the effect of PATH and of cortical extract was unexpected. Dougherty & White (1945) comment that degenerative changes in lymphoid tissue (mice and rabbits) 'were found within an hour following injection of either adrenotropic or adrenal cortical hormones and persisted for a 6 hr. period', and did not draw any distinction between the effects of hormone or cortical extract.

In the present observations, although the number of experiments was small (Table 2), the differences between the effects of the two substances was constant and definite (Pl. 2, figs. 5 and 7). These results are in conflict with those of other workers, but there are so many unknown factors involved that it is not possible satisfactorily to account for the discrepancy. There may, for example, be species differences. Thus Dougherty & White (1945) noted a marked difference between the reaction of lymphoid tissues in mice and rabbits. 'Within 24 hr., the lymphoid tissues of the mice resembled those in normal animals' (after giving PATH or cortical extract) 'except for the thymus which was still depleted of lymphocytes. In the rabbits, the degenerative changes recurred at 24 hr. after hormone injection.'

The dose and the time factor may also be important. In the present experiments the rats were killed 24 hr. after the last injection of hormone or extract, whereas Dougherty & White (1945) noted the maximal degenerative changes in mice within 6 hr. following injection. This would not explain in the present experiments the difference in reaction to PATH and cortical extract, though it may account for the failure to observe the more pronounced degenerative changes which they describe. For assuming that the immediate effect of cortical extract in the dosage employed is injurious, the lymphoid tissue may have time, if there is a sufficient interval between the doses of extract, and the damage not too severe, not only to recover but even to overcompensate for the injury and undergo hypertrophy. This type of lymphoid tissue reaction has been noted following the slight destructive action of small doses of X-rays (Drinker & Yoffey, 1940).

Another possibility is that PATH stimulates the cortex to secrete a much larger amount of hormone—or hormones—than is contained in the dose of cortical extract given. This, in the present state of our knowledge, can only be surmise, for we are not able to effect a quantitative correlation between the dose of PATH and the amount of cortical hormones secreted in response. In addition, there may be qualitative differences between the artificially prepared extract and the natural hormones. In two experiments in which both PATH and cortical hormone were given in conjunction, the cortical extract inhibited the customary depressant action of the PATH (Pl. 2, figs. 5 and 6; Pl. 3, figs. 9 and 10).

The weight changes in the experimental animals (Table 2) introduce a further factor for consideration. In two PATH experiments (nos. 44 and 45) the animals lost weight, whereas in the third (no. 46) there was a gain in weight. In the remaining five experiments there was also a gain in weight. In all the animals of this series (Table 2) the general condition seemed excellent; the animals were lively and active, and took their food well. Nevertheless the loss of weight in nos. 44 and 45 is quite marked. From the work of numerous observers (see review by Jackson (1925)), it is clear that the lymphoid tissues are very sensitive to inanition and malnutrition. In the present experiments it may be significant that the atrophic changes in lymph nodes were more pronounced in experiments nos. 44 and 45, in which loss of weight was marked, than in no. 46, where the animal gained weight. It is worth noting that this animal (no. 46) received the highest dose of PATH.

Macrophages in lymph nodes. Dougherty & White (1945) described the appearance of increased numbers of large macrophages in both thymus and lymph nodes after the injection of PATH or cortical extract. 'The macrophages were so numerous that under low power the cortex of the thymus possessed a pitted appearance.' In the present experiments this appearance of 'pitting' was found in the rat thymus after PATH (Pl. 3, fig. 12) and cortical extract (Pl. 3, fig. 13) but also quite appreciably in the normal thymus (Pl. 3, fig. 11). It is also well marked in lymph nodes after cortical extract (Pl. 2, fig. 7), and the macrophages then seem to stand out more clearly than in the normal node (Pl. 1, figs. 1 and 2; Pl. 2, figs. 7 and 8). Dougherty & White (1945) interpret the presence of these macrophages as being chiefly in response to the increased destruction of lymphocytes which they describe as occurring. But this interpretation is not altogether convincing. It is quite true that since Flemming (1885) first described these macrophages, and noted the stainable bodies which they contained (his 'tingible Körper'), it has been accepted by most workers that these are ingested lymphocytes. Pl. 1, fig. 2, and Pl. 2, fig. 8, illustrate clearly the presence of several macrophages and their stainable bodies. But in the present experiments the macrophage increase was most noticeable in the animals given cortical extract, even though there was no indication of increased lymphocyte destruction, with the exception of the animals which were given colchicine in addition. In these latter (Pl. 1, fig. 4) the usual karyoclasic phenomena were noted, whereas in all other animals (Pl. 1, fig. 2; Pl. 2, figs. 6 and 8;

Pl. 3, fig. 10) the overwhelming majority of the small lymphocytes appeared normal.

Furthermore, there are always some macrophages present in normal lymphoid tissue, and they become much more abundant when it is undergoing active proliferation. 'In practically all secondary nodules in the active stage there are a fair number of cells of macrophage character' (Bloom, 1938), and one might add not only in the secondary nodules, but also in the diffuse lymphoid tissue (see also Latta, 1921). In the light of these facts it is interesting to note that it was more especially in the rats which were given cortical extract alone that the macrophages were particularly in evidence, and it was precisely in these animals that the lymphoid tissue was unusually active, as shown by the large and packed germ centres, containing numerous mitoses, and the dense diffuse lymphoid tissue occupying most of the node (Pl. 2, figs. 7 and 8).

If the stainable bodies in the macrophages are ingested lymphocytes, as appears likely, it is probable that there are always a few degenerating lymphocytes even in normal lymphoid tissue, and their number increases with the increase in the total number of lymphocytes which occurs when lymphoid tissue undergoes active proliferation.

The level of the blood lymphocytes. The degree of activity of the lymphoid tissue might be expected to influence the lymphocyte content of the blood, and reference has already been made to the observation by numerous workers of lymphopenia after the administration of PATH or cortical extracts. The degenerative changes observed in lymphoid tissues seem to point to diminished lymphocyte production being the cause of this lymphopenia. In the terminology of Drinker & Yoffey (1940), where X is the number of newly formed lymphocytes entering the blood, and Y the number leaving the blood, the number actually present in the blood at any time depends on the balance between X and Y, and if $X/Y=1{\cdot}0$ then the level of the blood lymphocytes will remain constant. In the case of PATH and cortical extract, the lymphopenia would appear to be of the $X-/Y$ type, if there are frank degenerative changes in the lymphoid tissues. However, it is possible that this is not the only factor involved. Thus in the present experiments the lymph nodes after repeated injections of cortical extract seemed to be hyperactive, yet the blood lymphocytes showed a distinct drop (Table 2). In these circumstances X is more likely to be $+$ than $-$, but a lymphopenia would still be possible if Y increased to an even greater extent than X. Or briefly, the lymphopenia would be of the $X+/Y++$ variety.

Dosage. Before concluding from any experiments with Path and cortical extract that the adrenal cortex regulates the activity of lymphoid tissue and the level of the blood lymphocytes, one has to consider how far the dose given corresponds with the secretion in the normal animal. As far as PATH is concerned, this question cannot yet be answered. But in the case of cortical extract the work of Vogt (1943) is of interest.

Vogt (1943) estimates the daily output of the cortex as equivalent to 2·3 c.c. of the particular cortical extract employed per 100 g. body weight, and states further that 'this figure is probably an underestimate'. The extract which she used cannot be directly compared with Eschatin, since methods of assay are different. But assuming that the extracts are of the same order of potency, the daily output of the suprarenal cortex would be equal to 115 dog units per 100 g. body weight per day. In the present experiments the dose of cortical extract given was very much smaller than this, ranging from 0·5 to 2·0 dog units per 100 g. body weight per day. This dose not only produced the changes in the lymphoid tissues and blood lymphocytes already noted, but also characteristic changes in the adrenal, such as the storage of birefringent material in coarse masses instead of the usual dust-like particles (Baxter, 1945). If Vogt's (1943) data are correct, it is surprising that these comparatively small doses should have such a definite effect.

Antihormones. In the experiments involving continued administration of PATH the question of antihormone formation should obviously be considered. We have at present no evidence to offer on this point.

SUMMARY

In healthy adult rats, and in the dose given, the subcutaneous injection of pituitary adrenotropic hormone (PATH) usually results in a lowering of the blood lymphocytes after 3–5 hr. Aqueous extract of suprarenal cortex has a similar effect. Continued daily injections of PATH or cortical extract or both in conjunction for a period of 4 weeks also usually tend to lower the blood lymphocytes, though the effect is more definite with cortical extract than with PATH. PATH given daily for 4 weeks produced regressive changes in lymph nodes and thymus, whereas cortical extract gave rise to a slight but definite hyperplasia of lymphoid tissue. Subcutaneous injection of colchicine is followed after 16–17 hr. by (*a*) widespread destructive changes in lymphoid tissue, (*b*) the appearance in the circulating blood of immature bone marrow cells, both myeloid and erythroid. In two rabbits, daily injections of aqueous solutions of suprarenal cortex for 16 and 40 days respectively produced no significant change in the blood lymphocytes.

The rabbit experiments were performed by one of us (J.M.Y.) while working in the Department of Anatomy, University College of South Wales and Monmouthshire, Cardiff, under Prof. C. M. West. We are indebted to Mr J. E. Hancock for the photomicrographs.

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EXPLANATION OF PLATES

All sections fixed in Zenker-formol, embedded in paraffin, cut at 6μ, and stained iron haematoxylin. All taken from rats.

PLATE 1

Fig. 1. Normal lymph node. Cervical. ×50.

Fig. 2. Part of a germ centre and some surrounding lymphoid tissue from fig. 1. Note mitoses, and also large macrophages containing stainable bodies. ×600.

Fig. 3. Animal 33. Table 1. Cervical lymph node 16½ hr. after subcutaneous injection of colchicine (0·2 mg./100 g. body weight). ×50.

Fig. 4. Germinal centre and adjacent lymphoid tissue from fig. 3. Node seems disorganized, and most of the lymphocyte nuclei show pyknosis and karyorrhexis. The overall picture is one of typical karyoclasic shock. ×600.

PLATE 2

Fig. 5. Animal 45. Table 2. Mesenteric lymph node, from an animal given daily injections of PATH for 28 days. In comparison with the normal (fig. 1), note the diminution in lymphoid tissue, the narrow cortical zone with small and poorly developed germ centres, the medulla with large and dilated sinuses. ×50.

Fig. 6. Germinal centre, from fig. 5, with zone of small lymphocytes and a dilated lymph sinus adjoining. Note mitoses in germ centre. Small lymphocytes perfectly normal. ×600.

Fig. 7. Animal 56. Table 2. Cervical lymph node after twenty-eight daily injections of cortical extract. Note the dense lymphoid tissue and well-marked germ centres. Compare with fig. 1 (normal) and note the slight increase in 'pitting', due to larger and somewhat more numerous macrophages. Contrast also with fig. 5 (PATH) and note the marked difference in reaction of lymphoid tissue to PATH and cortical extract. ×50.

Fig. 8. Part of fig. 7. Note well-marked germinal centre, with numerous mitoses, and also several macrophages. This is a much more active germ centre than fig. 6 (PATH), and somewhat more active than fig. 2 (normal). ×600.

PLATE 3

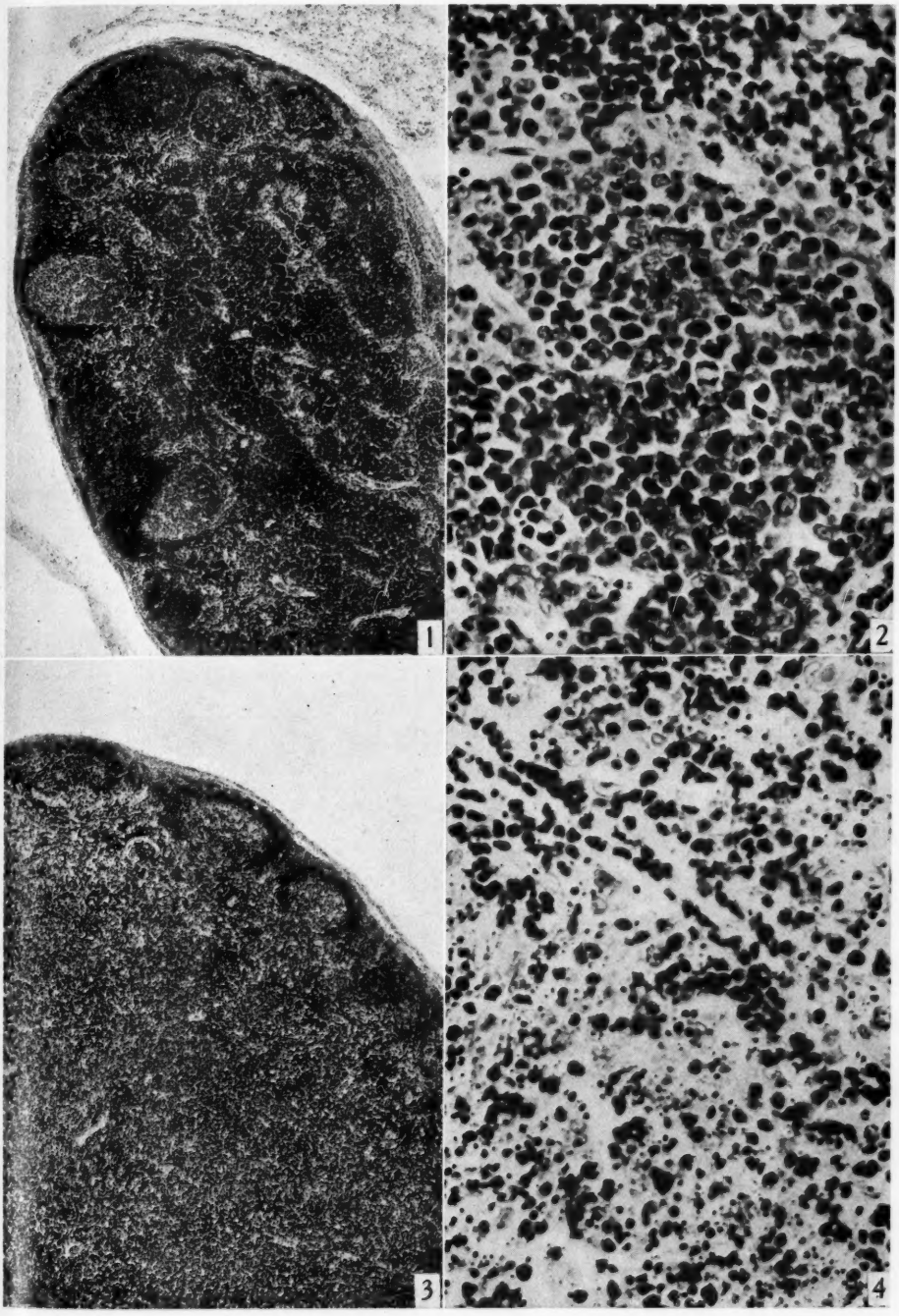
Fig. 9. Animal 51. Cervical lymph node, from a rat injected with both PATH and cortical extract for 28 days. Compare with fig. 5, and note that the cortical extract seems to have diminished the effect of the PATH. ×50.

Fig. 10. Part of fig. 9. Note the active germ centre with numerous mitoses. ×600.

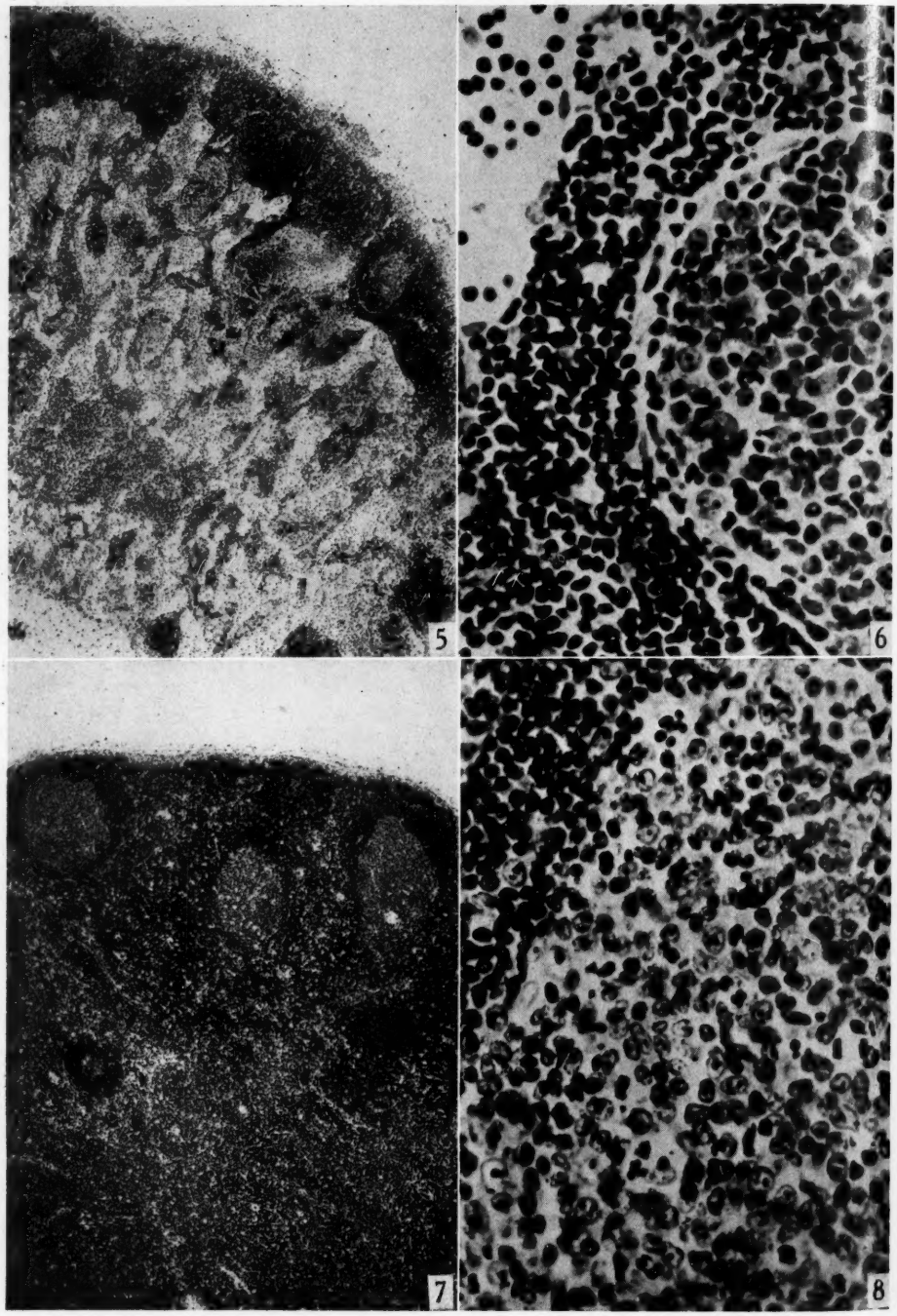
Fig. 11. Normal rat thymus. Note the 'pitting' normally present. ×50.

Fig. 12. Animal 46. Thymus of rat injected with PATH for 28 days. Compare with fig. 11 and note the diminution in cortical area, and increase in medulla. ×50.

Fig. 13. Animal 56. Thymus from rat treated with cortical extract for 4 weeks. Cortex dense, packed with small lymphocytes. Compare with fig. 12. ×50.



YOFFEY AND BAXTER—SOME EFFECTS OF PITUITARY ADRENOTROPIC HORMONE



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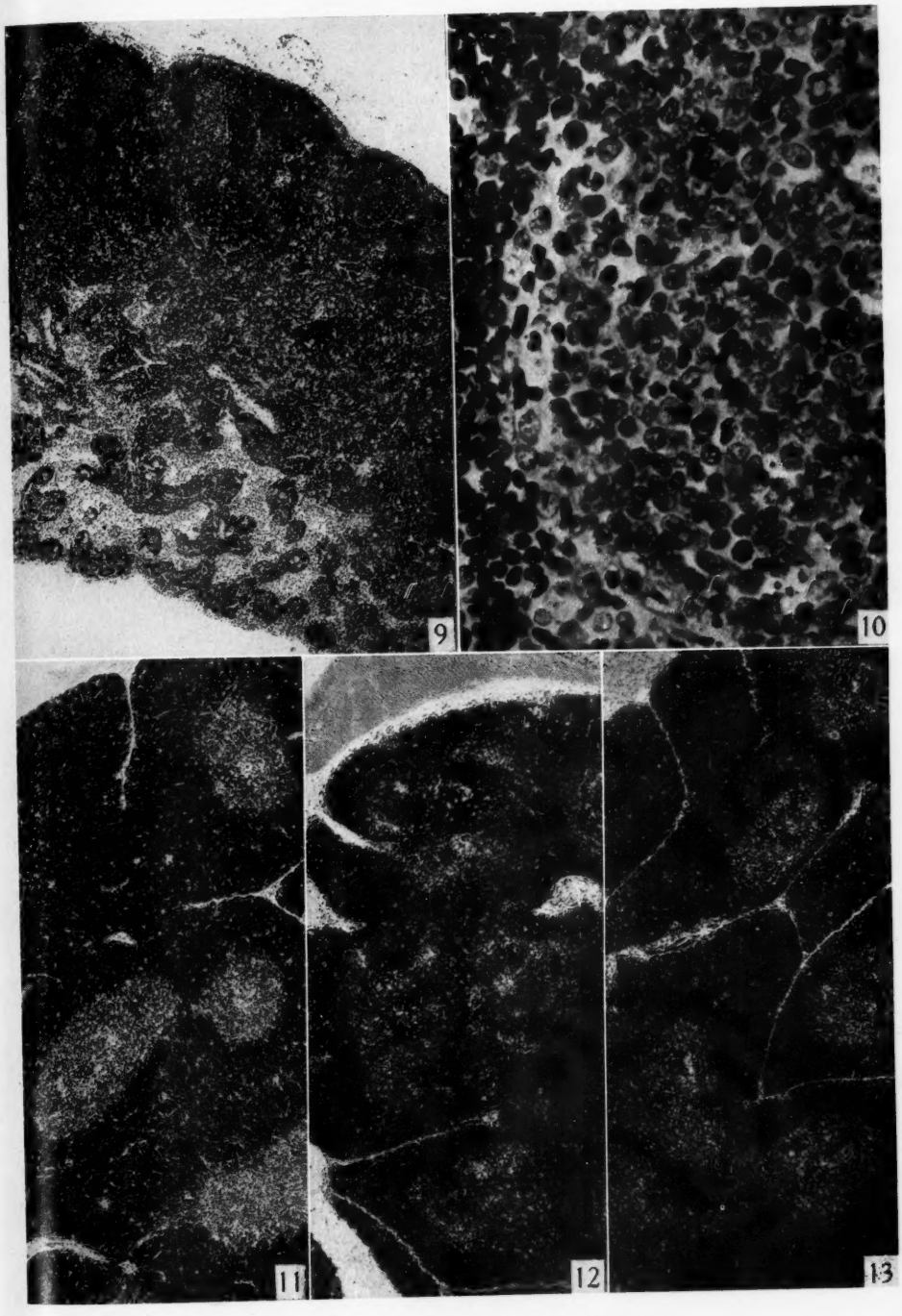

YOFFEY AND BAXTER—SOME EFFECTS OF PITUITARY ADRENOTROPIC HORMONE

UNIV. OF MICH.

THE GROWTH CYCLE OF THE CELLS OF THE ADRENAL CORTEX IN THE ADULT RAT

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The experiments reported in this paper have been designed to test the claim that cells of the capsule of the adrenal gland become transformed into glandular elements, thus contributing new cells to the cortex to replace those disintegrating on completion of their life cycle. It seems generally agreed that the adrenal cortex of mammals shows centripetal migration of cells. Growth and differentiation take place in the outer parts of the cortex—the zona glomerulosa and the outer portion of the zona fasciculata; mature cells are to be found in the spongiocyte region of the zona fasciculata, while the inner zona reticularis is a place where cell death and disintegration occurs (Graham, 1916; Hoerr, 1931; Bennett, 1940). To this, Zwemer and his co-workers (Zwemer, Wotton & Norkus, 1938; Salmon & Zwemer, 1941; Wotton & Zwemer, 1943) have added the concept that a stratum of undifferentiated 'fibroblast-like' cells in the deeper part of the capsule normally elaborates cells which, by proliferation and migration, become glandular elements of the zona glomerulosa. It has further been suggested that the capsule plays an important part in regeneration of the gland after enucleation (Ingle & Higgins, 1939; Baker & Baillif, 1939).

Salmon & Zwemer (1941) studied the problem of transformation of capsular cells into glandular in animals stained *intra vitam* with trypan blue. They noted that cells in the capsule of the adrenal stored trypan blue in the form of fine granules after subcutaneous injection of the dye, and they then traced the subsequent fate of these marked cells. They concluded that, with increasing time interval after injection, there was a progressive inward movement of dye-containing cells until at 20–30 days after the first injection of dye, blue cells were to be found in the zona reticularis. Analysis of the data presented by Salmon & Zwemer raised some doubt as to the correctness of the interpretation that had been made. Not only did the amount of dye given to the animals vary, but so also did the period of time during which it was administered. Since, as Cappell (1929) has shown, vital dye is found in high concentration in the blood plasma for several days after a single injection, the time during which cells were exposed to the dye was considerable in the experiments reported. This indicates that it is difficult to determine just when any particular group of cells was first 'marked' by the dye. Further, in the experiments of Salmon & Zwemer, where dye-containing cells were observed in the zona reticularis 20–30 days after the first injection, it appears that injection of dye was continued throughout the whole of this time, and that the appearance might be, as Calma & Foster (1943) suggested, an expression of generalized absorption of dye by the cortical cells. Calma & Foster were unable to confirm the results of Salmon & Zwemer and in view of the difficulties in the acceptance of the latter's views, it was felt that their work should be repeated using a rigidly controlled technique. The amount of dye given required to be below the toxic level and to be constant with respect to the body weight of the animal. This predetermined amount should be given in one dose, thus ensuring that the 'commencement' of the experimental period can be dated as accurately as possible. Such an experimental series is described in this paper.

If it be true that the life cycle of the cortical cells begins in the capsule, and the cells then migrate inwards, it is possible that the mitotic activity of the capsular and subcapsular cells might give an indication of this.

Bearing in mind the observations of Graham (1916) and Hoerr (1931) that mitotic figures were most numerous in the outer part of the adrenal cortex in guinea-pigs, it seemed that determination of the numbers of these in the various cortical zones (including the capsule) of the rat, might be of value in two ways. First, determination of absolute values for dividing cells in the different cortical zones should give information regarding the relative amount of proliferation in each, especially if colchicine were used to arrest mitosis over a definite period of time. Secondly, such absolute values for normal rats would permit comparisons of the amount of cell proliferation in the adrenal cortex following experimental procedures. This second line of inquiry is not considered further in this paper. We know very little about the rate of cell division in the normal rat adrenal, and such information would be of obvious use when we consider how much the rat is used in endocrinological research.

Blumenfeld (1939) and Nathanson & Brues (1941) have reported on the number of dividing cells in the rat adrenal cortex. The former expressed his results in terms of mitoses per cubic mm. of cortex, while Nathanson & Brues (who worked with immature

* Aided by a grant from the Colston Research Fund.

female animals), gave their observations as the number of dividing cells per 10,000 cells counted. In the experiments reported here, the method of Nathanson & Brues has been amplified, absolute figures being given for the entire cortex, and for each zone of it, including the capsule.

MATERIAL AND METHODS

Twenty-six adult female albino rats of the Wistar strain were used in this work. All were kept under identical conditions of diet and housing. Special consideration was given to the temperature of the animal house, which was maintained at 70° F. to avoid any possible stimulant effect of low temperature on the adrenal cortex.

The trypan blue used for injection was made up in a 1 or 2% solution in distilled water. It was filtered and stored in 2 c.c. ampoules, which were then sterilized. This was found to be a very convenient method of keeping the dye solution, and, contrary to what has been said by some (Romeis, 1932), no untoward effects were observed on injection of the dye solution thus made, 4 months or more after preparation. The animals were stained by a single intraperitoneal injection of either the 1 or 2% solution of the dye in such amount that a dosage of 20 mg./100 g. body weight was given. It had been determined by preliminary experiment that this amount of dye was far below the toxic level and yet gave adequate staining of adrenal cortical cells.

Since Cappell's (1929) observations had shown vital dye to persist in the blood plasma for some time after a single injection, it was necessary, for the evaluation of our results, to investigate this point further. Accordingly an adult rat was given a single injection of the standard dose of trypan blue for its body weight. The blood plasma was examined at 24 hr. intervals afterwards. This was coloured deep blue until the 5th day, when a slight lessening of the colour was observed. On the 6th and 7th days this lessening of blue colour in the plasma became much more obvious. The experiment was then terminated. Although no quantitative estimations were made, it may be stated that a single intraperitoneal injection of trypan blue results in a uniformly high concentration of that dye in the plasma for 5 days; after that time the concentration diminishes fairly rapidly.

Animals were killed by ether at intervals from 12 hr. to 21 days after injection. The adrenals were fixed in Bouin's fluid, Heidenhain's Susa fluid, 10% formol-saline or Bensley's formalin-Zenker. For preservation of dye granules, the first two of these fixatives were found extremely good. Formalin-Zenker was quite useless. Paraffin sections were cut at 4–5 μ. When the object was to study dye granules, sections were mounted unstained, or lightly counterstained with Mayer's carmalum or safranin. Safranin gave very beautiful preparations in which the cell cytoplasm had a somewhat translucent appearance and the dye granules stood out in vivid contrast.

In the investigation of cell division in the adrenal cortex, two series of glands were used. One consisted of 14 adrenals from normal animals, and the other of seven glands from rats which had received colchicine in a dosage of 0·15–0·2 mg./100 g. body weight by subcutaneous injection 16 hr. before death. The colchicine must be freshly prepared, since in solution it undergoes rapid deterioration under the influence of light. The adrenals from both these groups were sectioned in paraffin at 5 μ and stained either with iron haematoxylin or Masson's trichrome stain (using light green).

OBSERVATIONS

(1) *The reaction of the cortical cells of the rat adrenal to intravitam trypan blue*

The reactions of the various zones of the cortex will be described separately, and for the purposes of this paper, the capsule is considered as a cortical zone.

Capsule. The adrenal capsule in the adult rat consists of some 4–6 layers of cells. The most peripheral are elongated and spindle-shaped in transverse section, and, as one passes towards the zona glomerulosa, the cells become shorter and plumper, the cytoplasm increasing in amount. A variable number of macrophages is found in the outer layers of the capsule.

Capsular cells commenced to segregate trypan blue a few hours after its introduction into the circulation of the animal. Twelve hours after injection a few, small, pale blue granules were visible in a number of the fibroblast-like cells here. Much larger pale blue granules could be seen in the macrophages at this time. The granules became more intense in colour, and rapidly increased in number. At 48 hr. the entire capsule was full of cells exhibiting these small, blue granules (Pl. 1, fig. 1). In all the cells of the capsule, except in the macrophages, the dye particles were characteristically small and of uniform size. Once established in the cell cytoplasm, they persisted throughout the experimental period, being found at all times up to 21 days after injection.

Glomerular zone. The glomerular cells reacted to trypan blue by segregating the dye in the form of very fine granules, exactly similar to the granules seen in the majority of the capsular cells. The segregation process commenced during the first 24 hr. after injection and seemed to occur with equal intensity at any level in the zone. Thus, at 30 hr. after injection, some glomerular cells as deep down as the outer border of the zona fasciculata showed dye in their cytoplasm, and at 48 hr. cells of the entire glomerular zone contained these granules (Pl. 1, fig. 1). In the preparations studied there was no suggestion of an inward spread of the

dye from the periphery of the adrenal cortex. Segregation of it occurred in a similar manner in all the glandular cells and about the same time after injection. In cells undergoing mitosis it was noted that the dye granules were dispersed in approximately equal numbers to the extremities of the spindle, thus presumably behaving as inert cytoplasmic structures.

Fascicular zone. *Intra vitam* trypan blue was not constantly observed in cells of the zona fasciculata. Most commonly the dye was found in cells at the outermost part of this cortical zone (Pl. 1, fig. 5), where small granules were found fairly evenly distributed throughout the cytoplasm of some of the cells. They resembled the granules in the cells of the glomerular zone and the fibroblast-like cells of the capsule, but differed in that they appeared somewhat later, about 4 or 5 days after injection. But, at this time, the concentration of trypan blue in the blood plasma was still high (see above).

Trypan blue was sometimes noted in cells in the deeper part of the zona fasciculata (Pl. 1, fig. 4). In these, the granules were much coarser than in the cells of the outer cortical zones. They therefore resembled macrophages, and their association with cortical sinusoids, as well as other micro-anatomical characters, left no doubt that they were phagocytic cells of the sinusoidal endothelium.

Reticular zone. A number of cells in the reticular zone constantly contained dye during the experimental period.

The first type recognizable (Pl. 1, fig. 3; Pl. 2, fig. 9) was an irregular cell whose nucleus was hyperchromatic, deformed and located at one side of the cell body. The cytoplasm contained vital dye along with masses of pigment. The amount of vital dye taken up by the cell seemed to vary inversely with the amount of pigment present. This dye was not arranged regularly in the cytoplasm, but appeared as masses of varying size between the pigment granules. These cells conformed in other characters with the 'dark' cells of the zona reticularis described by Hoerr (1931).

A second type of dye-containing cell constantly found in the zona reticularis is illustrated in Pl. 1, fig. 2. It was found to be characteristic of this cell that trypan blue had entered into it, and stained the cytoplasm in a *diffuse* manner. Sometimes even the nucleus took up the vital dye. In the group of cells figured there are all stages between mere coloration of the cytoplasm by trypan blue to intense staining of the nucleus along with vacuolar degeneration of the cytoplasm. For reasons which will be discussed later, these cells must be considered as dying or dead.

(2) *Cell division in the normal adrenal cortex*

As already noted, the number of mitotic figures observed in each cortical zone was determined in relation to the total number of cells counted in that zone. Tables 1 and 2 give the results obtained from two series of animals; the first consisted of normal individuals, while the rats in the second group had received colchicine.

Table 1. *Mitotic counts in adrenals from normal rats. Expressed as dividing cells per* 10,000 *cells counted*

Animal no.	Capsule	Glomerulosa	Fasciculata	Reticularis	Total cortex
194A	17	23	0	0	10·6
194B	15	17	33	14	18·9
195A	33	17	0	19	16·7
195B	19	10	7	7	10·1
196A	25	46	20	16	26·2
196B	16	24	38	7	22·1
199A	20	21	8	0	11·8
199B	17	16	6	0	8·8
203A	11	0	13	9	9·1
203B	0	16	24	0	10·0
204A	7	8	0	0	3·6
204B	0	0	6	6	3·3
205A	0	22	7	0	7·1
205B	7	16	17	0	10·0
Average	13·3	16·8	12·7	5·6	12·0

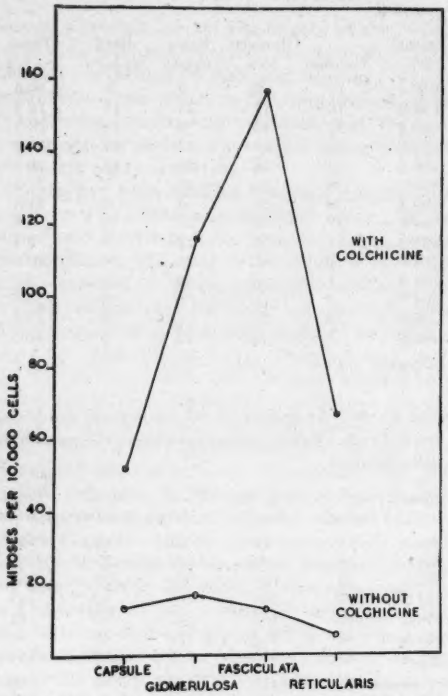
Table 2. *Mitotic counts in adrenals from colchicine-treated rats. Expressed as dividing cells per* 10,000 *cells counted*

Animal no.	Capsule	Glomerulosa	Fasciculata	Reticularis	Total cortex
208A	45	46	83	46	64
208B	54	89	133	37	80
233A	56	151	231	93	133
233B	25	169	212	82	118
238A	62	105	143	53	91
240A	66	104	120	48	86
242A	55	152	179	102	124
Average	52	116	157	66	99

It will be seen from Table 1 (data from fourteen adrenals) that the mitotic count for the total cortex varied, in the normal animal, between 3·3 and 26·2 per 10,000 cells. The average for the series was 12·0. In the individual zones of the cortex there was variation between different animals and even between the two glands from the same animal. Dividing cells were most numerous in the zona glomerulosa where the average for the series was 16·8 per 10,000. They were rather less frequent in the capsule (average, 13·3), and slightly less frequent still, in the zona fasciculata, where the average was 12·7. These figures are graphically represented by the lower curve in Text-fig. 1.

For the second series it was calculated from the data of Brues & Cohen (1936) that 0·15–0·2 mg. of colchicine per 100 g. body weight would be effective in arresting cell division over 16 hr., and the adrenals from rats thus treated showed many more mitoses than normal. Thus, in Table 2, we find the

average number of mitoses for the whole cortex to be 99 per 10,000 cells. The variation is not so marked as in the normal animals, ranging from 64 to 133. The highest mitotic rates were found in the zona fasciculata; the zona glomerulosa came next, while in the zona reticularis and the capsule the rates were considerably lower (Text-fig. 1, upper curve).



Text-fig. 1. Frequency curves of mitoses for the cortical zones of the adrenal in normal and colchicine-treated rats.

It is worth noting that the distribution of mitoses in the various cortical zones was not uniform in the adrenals studied. To illustrate this, camera lucida tracings (Text-figs. 2, 3) were made from sections through the centre of the adrenal in two colchicine-treated rats, and the positions of dividing cells plotted. In Text-fig. 2 there is great activity in one-half of the section while the other is relatively quiescent. In the active portion of the gland mitoses are numerous at all levels in the cortex, and it seems possible that the call for production of new cells varies in different parts of the same gland. There is, of course, nothing new in the concept of differential activity in various parts of one gland, but it does not hitherto seem to have been noted in the adrenal cortex.

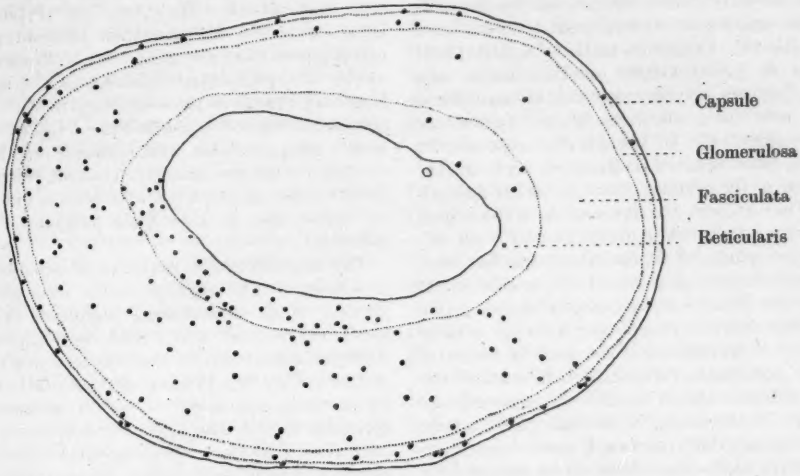
Text-fig. 3 demonstrates the conditions found in another animal. Here, mitoses are seen at all levels in the cortex, and in all parts of the section, but there is a tendency for them to be located more in the superficial than in the deeper parts of the cortex.

It is noteworthy that mitoses were observed in the zona reticularis in a considerable number of normal animals, and constantly in the animals treated with colchicine. Examples of such mitoses are shown in Pl. 1, figs. 2, 3, and Pl. 2, fig. 8. These are in glandular cells, and multiplication of endothelial and connective tissue elements (which occasionally is found in normal adrenals) has been disregarded in compiling Tables 1 and 2.

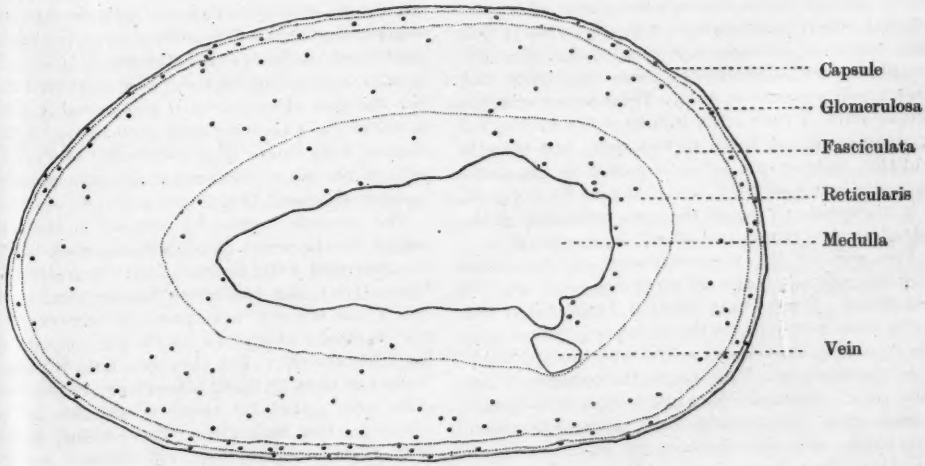
Mitotic figures in the adrenal capsule are shown in Pl. 2, figs. 6, 7, and it will be seen that they occupy a position in the capsule just external to the zona glomerulosa. It was not common to observe mitoses in the more superficial flattened cells of the capsule. Sometimes it was difficult to determine whether a dividing cell in the deeper region of the capsule should properly be assigned to that zone or to the zona glomerulosa. At some places (Pl. 2, fig. 7) there were appearances suggesting that cell cords of the zona glomerulosa were connected with, and taking origin from, germinal foci in the deep part of the capsule.

DISCUSSION

The observations on animals stained *intra vitam* do not support Salmon & Zwemer's contention that the administration of vital dye provides a *method* whereby the derivation of adrenal cortical cells from the capsule may be demonstrated. I do not deny the possibility of such an origin for zona glomerulosa cells, but submit that this method does not demonstrate it. In the present experiments, pale blue granules were observed in cells of the adrenal capsule 12 hr. after injection of dye; similar granules were visible at all levels in the zona glomerulosa 24–30 hr. after injection, while at 48 hr. the segregation of trypan blue by the cells of both capsule and glomerulosa was very evident (Pl. 1, fig. 1). It is extremely difficult to believe that, in this short time of 24 hr., such a number of dye-containing cells in the zona glomerulosa could have been derived from similar dye-storing cells of the capsule. The appearances suggest, on the contrary, that the fibroblast-like cells of the capsule, the cells of the zona glomerulosa and the cells of the outermost part of the zona fasciculata react simultaneously in a similar fashion to *intra vitam* trypan blue which is present in high concentration in the blood plasma during the whole of this period. In brief, the fact that the dye particles in all these cells (*a*) are morphologically similar, (*b*) become microscopically visible within 24 hr., and (*c*) show little evidence of diminution in number as one progresses inwards from the capsule, indicates that there is a certain functional affinity between these peripheral cortical cells, but no more than that. Calma & Foster (1943),



Text-fig. 2.



Text-fig. 3.

Text-figs. 2, 3. Camera lucida drawings of sections through the middle of the left adrenal of two adult rats treated with colchicine 16 hr. before death. The different zones and the sites of mitotic figures are shown.

repeating the experiments of Salmon & Zwemer (1941) for rats, were unable to satisfy themselves that the method yielded any evidence for centripetal growth of cells in the adrenal cortex. Similar findings were noted in the mouse by McPhail (1944).

The reactions to trypan blue shown by the cells of the zona reticularis do not seem to have been reported hitherto. Certainly, neither Cappell (1929) nor Calma & Foster (1943) mention them, and Salmon & Zwemer only saw dye-containing cells in the zona reticularis after 20–30-day periods of continuous injection. In the present experiments these *intra vitam*-stained cells were seen in the deepest zone of the adrenal cortex in all the animals studied. That an acid azo dye such as trypan blue, injected *intra vitam*, will enter into and stain diffusely the cytoplasm of degenerating cells has been known for some time. Ludford (1933) has discussed this phenomenon in his review of vital staining. He considers that diffuse cytoplasmic staining, with or without *intra vitam* staining of the nucleus, indicates cell senility and death; further, he believes that the degree of diffuse staining (in chronic experiments) is a measure of the decay of the cell. Relevant to this, Darlington (1937) perfused surviving organs with relatively high concentrations of trypan blue (1/4000 in Ringer-Locke solution) to detect cell death, while Bennett (1940) injected large amounts of vital dye for the same purpose in acute experiments. In both these procedures only the nuclei of dead cells were coloured by the dye. Pl. 1, fig. 2 shows cells in the juxta-medullary zone of the rat adrenal which have stained diffusely with trypan blue following a single injection of the dye. The morphological characters of these cells show that they are degenerate or dying. Their presence in the deeper part of the cortex indicates the undoubted occurrence of cell death in this zone, but since in addition mitoses are often observed in glandular elements here (see Pl. 1, figs. 2, 3 and Pl. 2, fig. 9), it is inaccurate to regard the zona reticularis of the rat adrenal as purely one of cell degeneration.

The cells of the zona reticularis that contain pigment and also take up vital dye must next be considered. Hoerr (1931) identified pigmented cells in the zona reticularis in the guinea-pig (where they are especially numerous) as the well-known 'dark' cells of this region. He came to the conclusion that they were glandular elements well on the way to degeneration. Blumenfeld (1939) has noted pigment cells in the zona reticularis of the rat adrenal, and described their increase in number and size after ovariectomy. Their nuclei resembled those of the cells lining the sinusoids, and this, with other evidence at his disposal, led him to believe them to be derived from the sinusoidal endothelium and to be phagocytic in nature. It has proved very difficult to decide in the normal material studied by the writer, whether the pigment cells of the rat adrenal are transformed glandular elements, or whether they belong to the reticulo-endothelial system. The fact that they are found in that part of the adrenal cortex where cell degeneration is undoubtedly taking place suggests their association with this process. They take up trypan blue in irregular coarse masses, thus resembling macrophages more than anything else, and indeed are not unlike the pigmented macrophages described by Rossman (1942) in association with the regressing corpus luteum of the macaque monkey. While it seems most probable that pigment cells in the rat adrenal cortex are mainly, if not all, reticulo-endothelial cells, the evidence available is not sufficient to enable one to pass final judgement on their nature.

The location of the majority of capsular mitoses in a layer of cells adjacent to the zona glomerulosa strongly suggests that some glandular elements, at least, are derived from these deep capsular cells. Additional evidence for this was seen in a number of sections (Pl. 2, fig. 7) where cell columns of the zona glomerulosa appeared to be in connexion with germinal foci in the capsule. Salmon & Zwemer (1941) refer to these deep capsular cells as a 'pre-glomerular zone' in their *intra vitam* stained animals. Our study of dividing cells in the rat adrenal capsule lends support to the concept of such a germinal zone for the gland proper.

The fact that mitoses occur with a higher frequency in both the zona glomerulosa and the zona fasciculata than in the capsule, need be no hindrance to accepting the view that some new glandular cells arise from the pre-glomerular zone. If such a cell divides, and a daughter cell is transported inward into the zona glomerulosa, it will probably continue to divide for a time depending on the need for new cortical cells—since it is commonly accepted that cells of the zona glomerulosa are functionally immature (Bennett, 1940).

The absolute values for mitoses in the adrenal cortex for the series of animals reported here may be compared with figures given by Nathanson & Brues (1941) for immature female rats, 4½ weeks old. These workers were primarily concerned with the effect of testosterone on the mitotic rate in the rat adrenal cortex, but they give data for controls. Values of from 23 to 42 mitoses per 10,000 cortical cells were found by them in animals to whom colchicine had been given. These values are lower than those reported here (99 mitoses per 10,000 cells of the entire cortex) but this may be due to differences in technique. Thus the dose of colchicine used by Nathanson & Brues was 0·1 mg./100 g. body weight; the time of death was never more than 12 hr. after administration of the drug. The maximum effect seems, from their figures, to have been obtained 4–10 hr. after giving the alkaloid. In the series reported here, on the other hand, both

the dose of colchicine (0·15–0·2 mg./100 g. body weight), and the time interval between injection and death of the animal (16 hr.), were greater. Brues (1936) claims that the number of mitoses seen in arrested metaphase after administration of colchicine over a period of time is equal to the number of mitoses which would normally have occurred and gone on to completion during that time. Brues & Cohen (1936) state that the time period when colchicine exercises its effect on dividing cells is from 6 to 18 hr. after injection, the optimum dose being 0·1–0·2 mg./100 g. body weight. This means that the higher dosage and longer period of action of the drug in our series might well account for the higher figures obtained.

As far as the age factor is concerned, since it is usually assumed that young rats show a higher mitotic frequency than adults, one would have expected the counts of Nathanson & Brues on 4½ weeks-old rats to be distinctly higher than those in the present experiments, in which adult rats have been used. That this is not the case calls for an explanation which cannot yet be given.

There is a point concerning the identification of mitoses arrested by colchicine which must be stressed. The typical colchicine-arrested mitosis shows the chromosomes dispersed throughout the cytoplasm in a characteristic manner. Such cells are easily identified; but in dividing cells which have been subjected to the action of colchicine for relatively long periods of time, the chromosomes are clumped. Examples of such mitoses are given in Pl. 2, figs. 7, 8, and unless well-differentiated iron haematoxylin preparations are studied under the oil-immersion objective many of these mitoses may be missed.

SUMMARY AND CONCLUSIONS

1. The reactions of the adrenal cortical cells to *intra vitam* trypan blue have been investigated in a series of rats.

2. Most of the cells of the peripheral part of the cortex, that is, the capsule, the zona glomerulosa and the extreme outer part of the zona fasciculata segregate trypan blue in the form of minute, intracytoplasmic granules; this commences during the first 24 hr. after injection, and reaches a maximum at 48 hr. There is no evidence that dye-containing cells migrate from the capsule into the zona glomerulosa: the whole region seems to react as a functional unit. The contention of Salmon & Zwemer (1941) that *intra vitam* staining of the adrenal provides a method for demonstrating the origin of glandular elements from the capsule has not been supported by the present work.

3. Certain cells related to the capillaries in the inner part of the zona fasciculata store *intra vitam* trypan blue in the manner characteristic of macrophages.

4. In chronic experiments, dead and senescent cells in the zona reticularis stain *intra vitam* in a *diffuse* fashion. Their cytoplasm, and in the case of dead cells, their nuclei, appear uniformly blue. The pigment cells of this zone segregate trypan blue like macrophages, and are believed to be reticuloendothelial cells, not glandular elements. The number of both these cells in the adrenal cortex is an index of the amount of cell destruction in the gland.

5. Figures are presented for the rate of cell division in the cortical zones in animals treated with colchicine, and in normal ones. There is evidence that certain cells in the deeper part of the capsule give rise to zona glomerulosa cells by mitotic division. These cells may thus be termed 'preglomerular' as was done by Zwemer and his coworkers. Attention is also drawn to the presence of mitotic figures in glandular cells of the zona reticularis. In the rat, at least, this zone is one where cell degeneration and new formation normally proceed side by side.

It is a pleasure to acknowledge my indebtedness to Prof. J. M. Yoffey, who suggested this problem to me in the first instance, and whose continued interest and advice have been of great assistance. I wish to thank Miss D. Davidson for her careful work in the preparation of the coloured Plate and Mr J. E. Hancock for the photomicrographs.

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EXPLANATION OF PLATES

PLATE 1

(These figures are all drawn at a magnification of ×1050.)

Fig. 1. Capsule and zona glomerulosa 48 hr. after a single intraperitoneal injection of trypan blue. The cells of these two zones have stored the dye in the form of fine granules. A macrophage with coarser granulation is seen in the outer part of the capsule. A glomerulosa cell in metaphase shows dye particles concentrated at the opposite poles of the cell. Safranin stain.

Fig. 2. Juxta-medullary zone 6 days after a single dose of trypan blue. The medulla is to the left-hand side of the field. A number of cells have taken up the dye in a diffuse fashion, and in one, the nucleus has been stained. These are dead or dying cells. Note the mitotic figure in the bottom right-hand corner of the field. Safranin stain.

Fig. 3. Zona reticularis. Note the presence of a number of pigment cells with shrunken, hyperchromatic nuclei. Two fuchsinophil cells are to be seen, and there is also a cell in mitosis. Masson trichrome stain.

Fig. 4. Dye-storing cells in the wall of a sinusoid of the inner fascicular zone. The dye granules here are definitely larger than those found in the cells of the capsule and glomerulosa and approach those of the macrophage in size (cf. fig. 1). Safranin stain.

Fig. 5. Outer fascicular region 21 days after administration of trypan blue. The cells of this part react to the dye in the same way as do those of the capsule and glomerulosa. The number of granules in each cell is, however, less. Safranin stain.

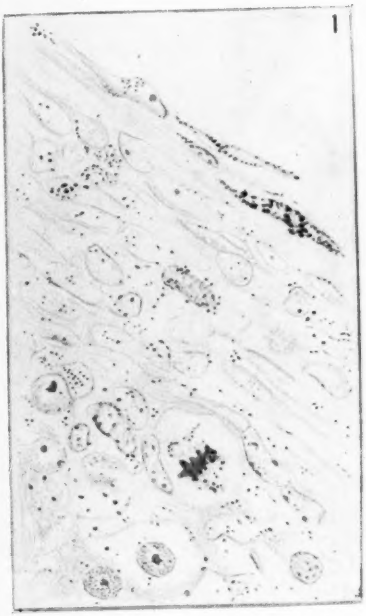
PLATE 2

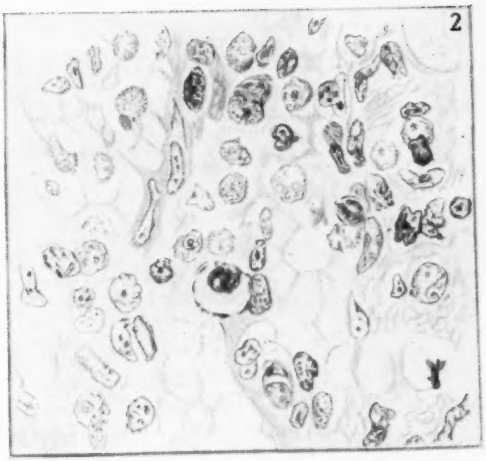
Fig. 6. Capsule and zona glomerulosa showing mitoses after colchicine. The periphery of the gland is towards the left. One mitosis lies in the 'pre-glomerular' region; a second is in the zona glomerulosa proper. Iron-haematoxylin stain. ×600.

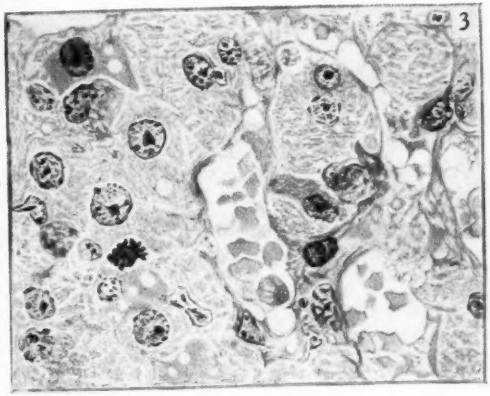
Fig. 7. Capsule, zona glomerulosa and part of the zona fasciculata from a rat treated with colchicine. There are two mitoses in the 'pre-glomerular' part of the capsule (upper part of photomicrograph); from the area between them a cord of gland cells extends into the zona glomerulosa. There is an example of the contracted type of colchicine mitosis in the zona fasciculata towards the bottom of the photograph. Masson trichrome stain. ×600.

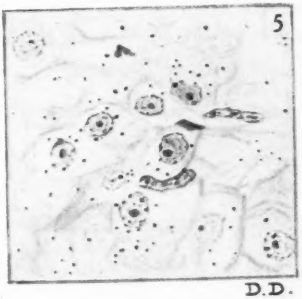
Fig. 8. Cortico-medullary junction of the adrenal from a colchicine treated rat. A mitotic figure with clumped chromosomes is seen about the middle of the photograph. A fuchsinophil cell (early degeneration) lies to its right, while some pyknotic nuclei of more frankly degenerate cells are below and to the left. Masson trichrome stain. ×600.

Fig. 9. Pigment cells (which appear dark in the photomicrograph) from the zona reticularis of a normal rat adrenal. Iron-haematoxylin stain. ×600.









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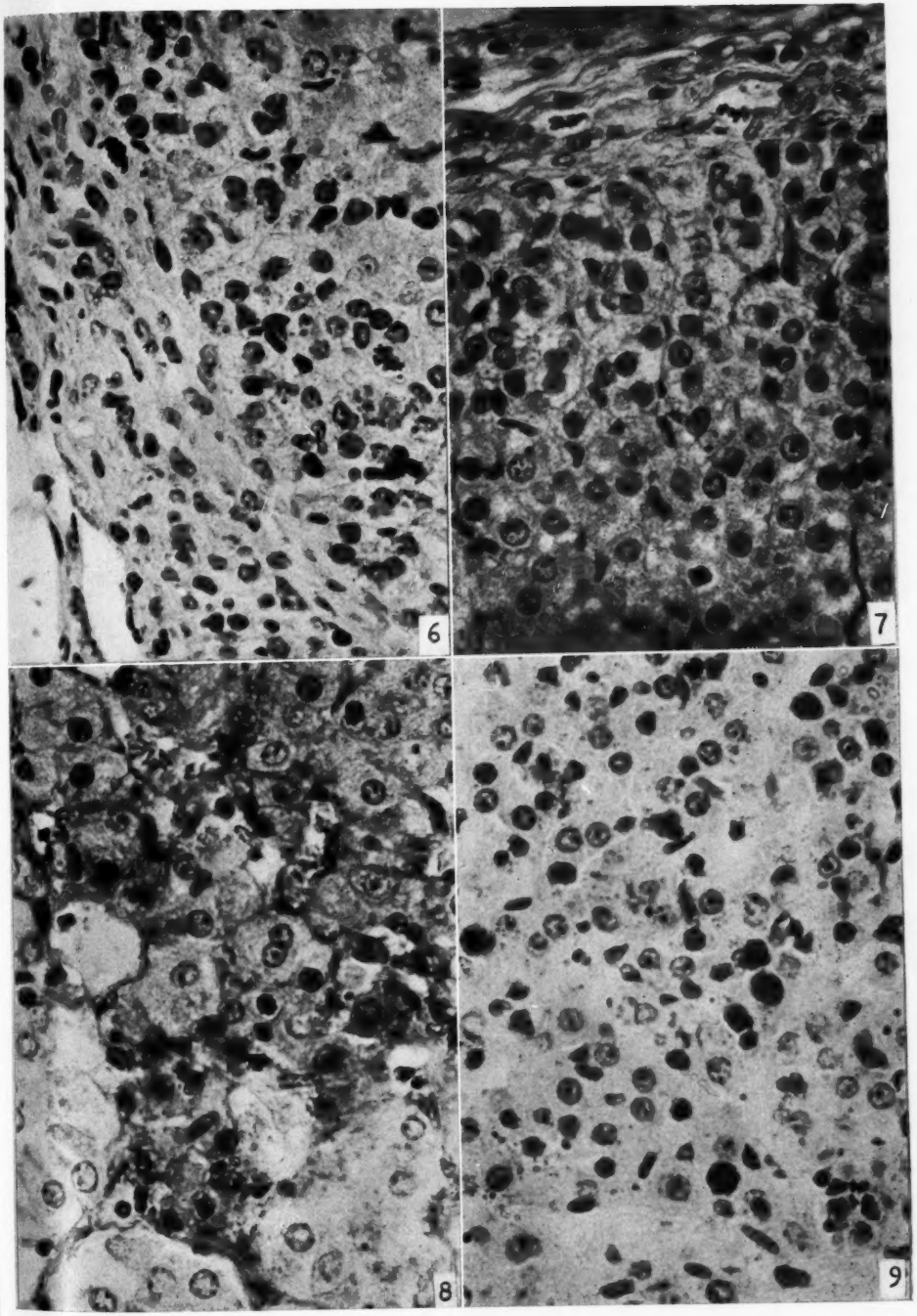

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THE BLOOD SUPPLY AND LYMPHATIC DRAINAGE OF TENDONS

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The surgical care of tendons presents many difficulties. Their behaviour as a tissue is largely dependent on the peculiarities of their blood supply, yet there is no available account of the anatomy and physiology of their vascular pattern. Kölliker (1850) said that tendons had practically no blood vessels; Schäfer, in Quain's *Anatomy* (1912, pp. 115, 343), gave a brief description of the blood vessels of muscle and tendon, and quoted Ludwig & Schweigger-Seidel (1872) as having described lymphatic vessels on the surface of tendons draining the muscle sheath. Mayer (1916) gave an outline of the vascular pattern and some details of the arrangement of mesotendons. The following study was undertaken to provide a fuller description of the vascular pattern of tendon.

MATERIAL AND METHODS

Flexor and extensor tendons, crossing the metacarpals and metatarsals, of cow, calf, adult human subjects, guinea-pig and dog were used. Indian ink or Winsor and Newton's water-colour vermilion cream was injected into blood vessels and lymphatics, and sodium nitroprusside-benzidine was used to delineate the blood vessels by Pickworth's method (1934). The Winsor and Newton water-colour cream gave a good pattern for the moment, but faded rapidly when treated with alcohol and clearing agents. Sodium nitroprusside-benzidine preparations were also suitable for immediate use only.

Blood vessels were injected via the metacarpal or metatarsal artery, or as they lay in the mesotendon, at a pressure of 100–250 mm. mercury. Lymphatics could only be injected satisfactorily in a disto-proximal direction, and it was sometimes necessary to make several attempts to get the needle into the superficial plexus. For this purpose it is best to use a no. 20 (S.-B.) needle with long bevel, to pass the needle as parallel to the tendon as possible, and to exert only gentle pressure. The warmer, fresher and younger the material, the better the results; old or cold tissue is unsatisfactory. Occasionally blood vessels are entered, but the pattern produced is different.

Double injection preparations were also made, blood vessels being injected with Indian ink and, following this, the lymphatics with vermilion cream. All preparations were verified by histological examination of injected areas, and in all cases the main lymphatic channel was traced back along the artery and vein to the carpo-metacarpal joint. In all cases but one there was no doubt that the injection had entered the lymphatics only; in one case both lymphatic and blood channel became filled with the injection medium, as was clearly proved under the binocular microscope. Injection preparations of either blood or lymph vessels alone are much more satisfactory than those in which both systems are injected.

OBSERVATIONS

The unit of tendon structure is the tendon fascicle. This consists of closely packed collagen fibres with their parent cells, the fascicles being bound together by a layer of fine connective tissue, the interfascicular tissue or endotenon. The cross-sectional area of the fascicle is variable but lies within certain limits (0·125–0·375 sq.mm.). The interfascicular tissue is probably largely nutritional in function, and the characteristic limit to the area of the fascicle is probably related to nutritional requirements. The interfascicular tissue may also be compared with the packing of a laminated spring and facilitates internal movement of the tendon. Where it has no synovial sheath the tendon is surrounded by loose fatty areolar tissue, the paratenon, which fills the interstices of the fascial compartment in which the tendon is situated.

In the simplest form of musculo-tendinous junction the muscle fibres are continued as collagen bundles, and each group of muscle fibres enclosed in perimysium continues as a tendon fascicle, the perimysium being continuous with the interfascicular tissue. Where the muscle fibres join the tendon obliquely this arrangement does not always hold, but there is a close correspondence between the grouping into bundles of muscle fibres and the grouping into tendon fascicles. At its insertion, the tendon becomes intimately connected with the bone and periosteum. The majority of the collagen fibres of the tendon are continued into the bone as the perforating fibres of Sharpey and the interfascicular tissue becomes continuous with the periosteum.

The tendon has a uniform structure throughout. The fascicles run from end to end, separated from each other and bound into a whole by connective tissue, continuous on the one hand with that of the muscle, on the other with the periosteum. The fascicles are roughly hexagonal in cross-section and show interfascicular grooves. In the interfascicular

tissue run blood vessels and lymphatics. The blood vascular system is simple and is uniform throughout the tendon, consisting of a series of longitudinal channels and a regular series of transverse anastomotic vessels (Pl. 2, figs. 4, 6).

Each fascicle is surrounded by a series of longitudinal arterial vessels, some of which can be seen in the interfascicular grooves on the surface of the tendon (Pl. 1, fig. 1). These longitudinal vessels are arteriolar in size, with a well-marked tunica media and a uniform lumen. They are fed from the surface vessels by transversely running channels of the same size which thread their way around and between the fascicles, linking the longitudinal vessels into a uniform plexus. Accompanying each arterial channel are two veins with the characters of large venules. They lie on each side of the artery, communicating frequently: their communication usually lies in the immediate neighbourhood of a branch or tributary (Pl. 2, figs. 4, 6).

Arising from the arterial bed is a series of capillary vessels which form loops draining into several capillary venules. This gives the impression of many more venous tributaries than arterial branches. The capillary plexus is intra-fascicular and does not appear to penetrate the collagen bundles. The loops may run from one longitudinal channel to another, or back to the parent channel.

Double injection preparations of blood and lymph vessels within the tendon were not satisfactory, but injections of lymphatics alone gave good results. These lymphatic channels follow the same course as the arteries. By the examination of stained sections of injected material the lymph vessels are seen to be closely associated with the blood vessels. The lymphatic plexus is quite characteristic and readily distinguished from the venous. It consists of several large vessels, usually two or three, of very irregular bore, with many interconnexions giving the appearance of a close meshwork surrounding the artery and veins (Pl. 2, fig. 5). The tributaries of the plexus are difficult to determine, but appear to be short, blindly ending capillaries lying in the interfascicular tissue and not extending very far from the main stem.

Connexions of this internal intratendinous blood and lymph vascular system with the external are located at four sites: (1) at the musculo-tendinous junction, (2) at the osteo-tendinous junction, (3) in the extra-synovial region, (4) in the intra-synovial region.

(1) *At the musculo-tendinous junction.* Each collagen fibre represents a muscle fibre. Each muscle fibre is surrounded by a capillary network fed from larger vessels lying in the perimysium. Where the muscle cell becomes a collagen fibre the capillary network ceases and of the vessels within the muscle only those of the perimysium continue into the tendon as the interfascicular vessels. These vessels, coursing from the muscle to the tendon, are the same size as those in the rest of the tendon, and there is no suggestion that the tendon derives much of its blood supply from the muscular arterioles. The vessels of the muscle sheath become continuous with those of the tendon sheath.

No deep lymphatic connexions across the musculo-tendinous junction have so far been displayed, but lymph vessels on the surface of the tendon can be traced into the muscle sheath, and a few into the perimysium. So far there is no satisfactory evidence that the deep lymph vessels of the tendon are continued into the perimysium, but the possibility exists.

(2) *At the osteo-tendinous junction.* Here the interfascicular vessels do not increase in size and do not receive any increased blood supply, but merely continue on their way, anastomosing with the rather scanty periosteal vessels. In some places branches may be seen passing through to the cortical layers of the bone. The vessels are small, few and irregularly distributed. Whilst the collagen fibres continue into the bone, the interfascicular tissue merges with the periosteum and takes its blood vessels with it. The vessels turn at right angles into the plane of the periosteum and become part of the periosteal network. The osteo-tendinous junction is not an important source of blood supply to the tendon. As with the musculo-tendinous junction, the fate of the lymph vessels in this region has not been ascertained.

(3) *Where the tendon has no synovial sheath* it is surrounded by very loosely woven connective tissue, so allowing considerable movement of the tendon; this has been called paratenon (peritenonium externum). Its function is that of an elastic sleeve which gives the tendon free movement against the surrounding tissue, at the same time maintaining its continuity with the tissues (see Mayer, 1916).

At frequent and regular intervals small vessels may be seen coursing through this areolar sleeve towards the tendon. They arise from vessels which lie in the vicinity and do not appear to have a specific origin and course. On approaching the tendon the vessels branch several times in the direction of the longitudinal axis of the tendon, lying in the interfascicular grooves. It is from these surface vessels that those within the tendon arise at regular intervals by transversely running branches threading between the fasciculi (Pl. 1, fig. 1). This loose areolar tissue and the layer of more condensed tissue around the tendon are continuous with the outer intermuscular tissue and the muscle sheath respectively. Vessels, of a size comparable with those approaching the tendon along its length, may be seen running in the muscle sheath and intermuscular tissue, becoming continuous with the surface vessels of the tendon. The paratenon is also continuous with

the loose areolar tissue on the surface of the periosteum, and surface vessels of the tendon are seen to pass off the tendon into this tissue. Some of these vessels may be quite large, e.g. those of the vincula brevia. Whilst the deep vessels are not large, the surface vessels of the osteo-tendinous and musculotendinous junctions may be significant in the maintenance of the nutrition of the tendon.

So far it has not been fully determined what happens to the lymphatic efferents on the surface of the tendon. There is no doubt that some of them pass alongside the entering arterial channels. Nothing has been found of their course at the osteo-tendinous junction except that relatively large lymph vessels can be seen running out in the loose tissue on the surface of the periosteum, apparently draining the area. It may be that they are communicating with the deeper channels of the tendon, and in view of the apparent lack of lymphatics in bone and periosteum it is reasonable to expect that the deeper lymphatics of the tendon drain to the surface vessels at the region of the osteo-tendinous junction, and from this surface network back along the main vascular channels of the region. This, however, remains to be demonstrated.

(4) *Where the tendon lies within the synovial sheath.* Within the synovial sheath the tendon has the same structure and vascular pattern as elsewhere. Instead of being surrounded by paratenon, the tendon is enclosed in a thick sheet of very vascular fibroelastic tissue, the visceral layer of the synovial membrane. The visceral layer is continuous with the parietal layer at the ends of the sheath, and the two may be connected by sheets or cords of tissue known as mesotendons. These mesotendons may be compared with the mesentery, and serve to convey blood vessels to the visceral layer, which is also vascularised by continuity from the parietal layer. The parietal layer is vascularised from the surrounding tissue.

In man mesotendons of the sheet type are found at the wrist and ankle, carrying several vessels often in a fan formation. Cord-like mesotendons are found in the digital sheaths where they are known as vincula longa, carrying one artery, two veins, and usually four lymphatics.

The synovial blood vessels show (1) a very fine capillary network on the surface of the synovial membrane, and (2) a deeper and much larger plexus of arteries and veins. The capillary network consists of fine vessels looping up to the surface from the arterioles and venules below. In some regions they may lie in small villi projecting from the surface. These loops are all of the same order of size; they are single and connect arterioles to venules (cf. Davies, 1946).

In the deeper layers of the synovial membrane lies the very rich plexus of larger vessels; it is with this plexus that the surface interfascicular vessels of the tendon communicate by short anastomotic channels, these short channels taking the place of the vessels of the paratenon.

The blood and lymph vascular systems of the synovial membrane will now be considered in more detail.

In the cow the deeper blood vascular plexus of the synovial membrane is virtually a continuous network of vessels, both arterial and venous. On the visceral layer there are direct anastomoses between the vessels in the various mesotendons, on the parietal layer between the large vessels entering the membrane, and round the ends of the sheath the larger vessels of the visceral and parietal layers anastomose. From this wide network of large vessels so formed, a series of loops arises, and from these loops, a subsidiary series of arcades, this process continuing until four to six generations of progressively smaller loops and vessels have been formed (Pl. 1, fig. 2). Each of these series of loops anastomoses with adjacent members of its own generation. Within the areas of each small arcade is a plexus of arterioles and venules from which arise the capillary loops of the surface layer.

The large vascular bundles contain a single arterial stem, branching only to form large vessels, and giving rise infrequently to small local vessels. This arterial stem is of fairly uniform bore within the limits of its own arcade and may be readily identified by this character. Running parallel with this artery, and placed one on each side of it, are two veins, one usually considerably larger than the other, and connected to each other by regular anastomosing channels of the same order of size as the larger. Both veins receive many local tributaries draining the perivascular plexuses previously mentioned. Anastomoses between the veins occur almost always at the entrance of a pair of tributaries. Examples of this may be seen in Pl. 1, fig. 2 and Pl. 2, fig. 6.

The two venae comitantes are evenly spaced from the central artery, and in preparations in which the blood vessels only have been injected the parallel venous channels are plainly seen, surrounded by a clear space. In this space runs a fine network of very small vessels which appear to be mainly venous and to drain regularly into veins. Arterial connexions cannot be seen in the injection preparations and are difficult to interpret on sections. The site of this plexus suggests that it may be perilymphatic, but this is difficult to make out from sections, and in double injection preparations of blood vessels and lymphatics, the fine plexus does not stand out clearly. In general the lymphatic vessels run with the blood vessels. From the periphery to the mesotendons they are multiple longitudinal channels,

acquiring valves shortly before they arrive at the mesotendon. In the mesotendon there is usually one artery and four lymphatics, but within a few centimetres of the mesotendon the four lymphatic channels fuse to a single vessel which may become double for short distances.

From the mesotendon to the periphery the lymphatic vessels are closely associated with the blood vessels. The larger lymph vessels have a corresponding pattern, lying always between the artery and veins, and on the outer side of the veins, so that each artery is accompanied by two veins and four lymphatics. These four lymph channels are intimately connected by a rich network of lymphatic capillaries, which pass on both sides of the blood vessels, enclosing them in a lymphatic plexus. This plexus follows the blood vessels in all their branching down to the smallest arcades (Pl. 1, figs. 2, 3). The terminations of the lymphatic capillaries appear to be short, blindly ending vessels with either convex or concave, slightly bulbous tips (cf. Davies, 1946). The concavity or convexity may well depend on the degree of distension of the vessel. The deeper plexus gives off these blindly ending lymphatic capillaries to the surface of the synovial membrane and communicates frequently with the interfascicular vessels of the tendon beneath.

The characteristic differences between lymphatic and blood vessels may be summarized as follows:

(1) Lymphatic capillaries are larger in bore than blood capillaries. This is true also of the more proximal vessels, and is probably due to the lack of muscle tissue in their walls.

(2) The lumen of the lymphatic vessel is irregular, giving it a moniliform appearance; the lumen of the blood vessels is regular.

(3) Rarely are lymphatics seen as single vessels; they are almost always in the form of a network of parallel vessels with frequent anastomoses producing a mesh with irregular spaces.

(4) The ultimate lymphatic vessels appear to be end vessels, i.e. they end blindly.

The actual site of the valves in the lymph vessels rests largely on deduction. Injection of lymphatic capillaries disto-proximally from the tendon is easy. Once a capillary lymphatic has been opened to the needle very gentle pressure of a few centimetres of water suffices to pour ink into the vessels, which fill rapidly along their course to the main channels. This free and rapid flow proximally from the tendon is constant. As well as a disto-proximal flow, there is always a local spread in all directions, adjacent channels fill and a large area of the synovial membrane can be injected from one insertion of the needle. Vessels can be seen running from the area to several mesotendons. This demonstrates that a lymphatic anastomosis exists, comparable with the blood vascular anastomosis and that no valves are present in the synovial network itself, neither are they seen under the binocular microscope, nor in histological preparations. This may be due to one or both of two factors: (1) if the lymphatic is not distended the valves are not readily visible, (2) if the lymphatic is distended with injection fluid, the valves are obscured.

In histological preparations, however, valves could be seen within a few millimetres of the mesotendon, in the mesotendon, and in the vessels proximal to this from the tendon. These facts agree with the results of attempted proximo-distal injection of lymphatics by inserting a needle into the vessels at the mesotendon or proximal to this. All these attempts have failed. No flow has occurred distally, and if the vessel has been clamped proximally, the vessel wall frequently breaks before the competence of the valves has been overcome.

We may assume therefore that valves do not exist in the synovial lymphatic network itself, but only proximal to this, and that here the valves are competent.

DISCUSSION

The literature abounds with papers on the nature of tendons, treatment of tenosynovitis, and the rupture of the extensor pollicis longus, but studies undertaken on the basis of the anatomy and physiology of tendons are rare.

For the surgeon, tendon has certain characteristics: (1) it appears to be practically avascular, (2) it is destroyed very slowly by inflammatory processes, (3) it may be cut and transplanted with impunity, (4) it is very difficult, if not impossible, to suture tendon within a synovial sheath without a high incidence of adhesion, (5) healing and sequestration of infected tendon is extremely slow.

In marked contrast to other tissues, tendon is virtually 'dead' during life. Almost the whole of the tendon is made up of collagen fibrils closely packed together, and apparently undergoing little or no metabolic activity. The living part of the tendon consists of the tendon cells lying between the bundles of collagen fibrils they have produced, and the interfascicular connective tissue. It is these living tendon cells which require a blood supply, but their proportionate volume is small, so that the vascular needs of the tendon as a whole are very small. Hence its apparent avascularity to the naked eye, and the scanty but regularly spaced plexus of vessels described above. It is interesting to note that of all the purely organic tissues of the body, tendon withstands the processes of decay longest. Because of the large proportion of 'dead' tissue, autolytic and bacterial destruction is slow. In the large sloughing ulcers of the foot which sometimes occur as a result of wet gangrene, the extensor tendons may be seen stretched across a bag of pus

and necrotic tissue, and whilst their surfaces are covered with purulent exudate, the central parts of the tendons show a relatively healthy appearance in cross section. Bacteria quickly gain a hold upon the connective tissue sheath of the tendon, and may penetrate a short distance down the interfascicular septa. This hold is shallow, however, and further penetration and extension of the eroding process is very slow.

Coupled with this slow rate of erosion of the tendon substance is the very slow rate of sequestration of dead material. Union of clean-cut tendon surfaces is a little slower than that of other connective tissues, but the process of organization of the fibrin film between the approximated tissues is not grossly delayed (Garlock, 1927; Mason, 1940).

The suture of tendon outside a synovial sheath is easy and, as the tendon is already adherent to the adjacent tissue, the functional result is good. Suture within a synovial sheath is difficult. Healing of the adjacent ends of the tendon is good, and the blood supply is virtually unimpaired as a result of the excellent longitudinal anastomoses and the frequent feeding of these by vessels from the synovia. But the methods of tendon suture at present in vogue do not consider the synovia as a separate structure, and no attempt is made to invert the edges of the synovia in the way that is customary in sewing a rent in the peritoneum. If this could be achieved it is possible that the incidence of adhesions might be reduced. The visceral synovia of the flexor tendons of the hand, however, is so thin and so firmly adherent to the underlying tendon, that this method of approach seems doomed to failure from the technical point of view.

Tendon may be transplanted or transposed with relative ease and the certainty that healing of the transplant will occur (Garlock, 1927). This is largely due to the low metabolic requirements of the tissue which can obtain sufficient blood supply from its longitudinal anastomoses until such time as vessels have developed in the new paratenon.

The arrangement of arteries, veins, and lymphatics in a tendon is interesting and typical and affords a further confirmation of the view that the lymph vessels tend to run in close proximity to the arteries (Harris, 1937). The tendon and its synovial sheath have one artery in the centre, on each side thereof a vein, and the lymphatic vessels disposed as four main channels one on each side of each vein and connected with one another along their whole extent by an intricate system of transverse vessels which surround the artery and veins. The veins are paired, with interconnecting branches in the immediate neighbourhood of the junction of tributaries, suggesting a mechanism for ensuring that the blood returns one way or the other and that venous pressure is evenly distributed.

There are no valves to be seen in the veins or lymphatics on the visceral synovial membrane, though they occur in the mesotendons. The flow of injection media is free in either direction in both the lymphatics and veins in the synovial membrane and in the vessels within the tendon substance. The arteries and their smaller branches have a thick wall of muscle and elastic tissue, and though no direct evidence of pulsation of these small vessels has been seen, it is reasonable to suppose that such pulsation does occur. Where valves are present in the lymphatics it is easy to visualize a pumping mechanism making use of transmitted pulsation from the artery. As a pulse wave passes through the artery, the lymph channel is compressed locally, and by means of the frequent valves, flow of lymph occurs in a disto-proximal direction from the periphery. Where valves are not present, the pulse wave produces local pressure, but the flow is not necessarily unidirectional. This pulse wave, much flattened in these relatively small peripheral vessels of the synovial membrane, may be of significance in promoting the flow of lymph to the mesotendons, where the valved pumping system takes over. The absence of valves and the rich anastomosis permits the easy flow of fluid towards any communication with the surrounding tissues, and so guarantees continuous good drainage in spite of any local pressure. There is evidence to suggest that lymphatic vessels contain no valves where they lie in or on a relatively firm, non-yielding tissue, but that valves occur where local tissue resistance is low, e.g. in loose folds of synovial membrane.

SUMMARY

1. A method of injecting the lymphatic vessels of the visceral synovial membrane of tendon sheaths is described.

2. The structure of the tendon, the musculo-tendinous junction and the osteo-tendinous junction is given, with their blood supply and lymphatic drainage.

3. The vascular pattern of the tendon and its sheath is described with special reference to their nutrition.

4. The observations are discussed in the light of the surgical pathology of tendons.

I wish to express my thanks to Prof. H. A. Harris, and Dr D. V. Davies for their continued encouragement and advice, and to Mr J. A. F. Fozzard for the photography of a difficult subject.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Digital extensor tendon of calf outside a synovial sheath. The blood vessels are injected with Indian ink. Note the arteries with their venae comitantes appearing from one side of the tendon, the longitudinal anastomoses formed in the interfascicular grooves, and the vessels running deep into the tendon from these. (×12.)

Fig. 2. Synovial membrane of superficial digital flexor tendon of calf. The blood vessels are injected with Indian ink. Note the arterial channels with their venae comitantes and the arcades of decreasing size which they form. (×12.)

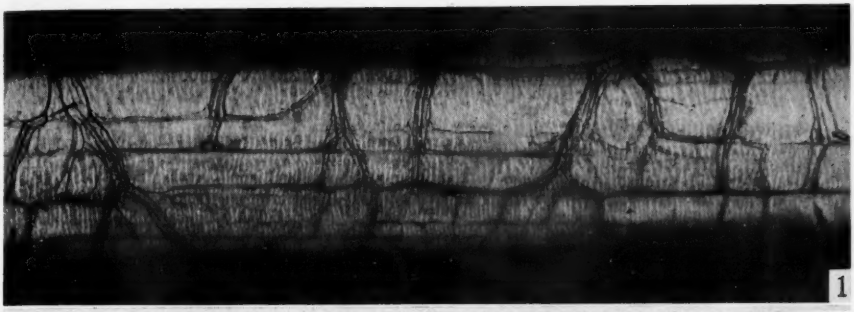
Fig. 3. Synovial membrane of superficial digital flexor tendon of calf. The area corresponds to that of fig. 2, and the lymphatic vessels have been injected with Indian ink. Note the arrangement of four parallel vessels with clear spaces between, where the artery and venae comitantes are to be found. (×12.)

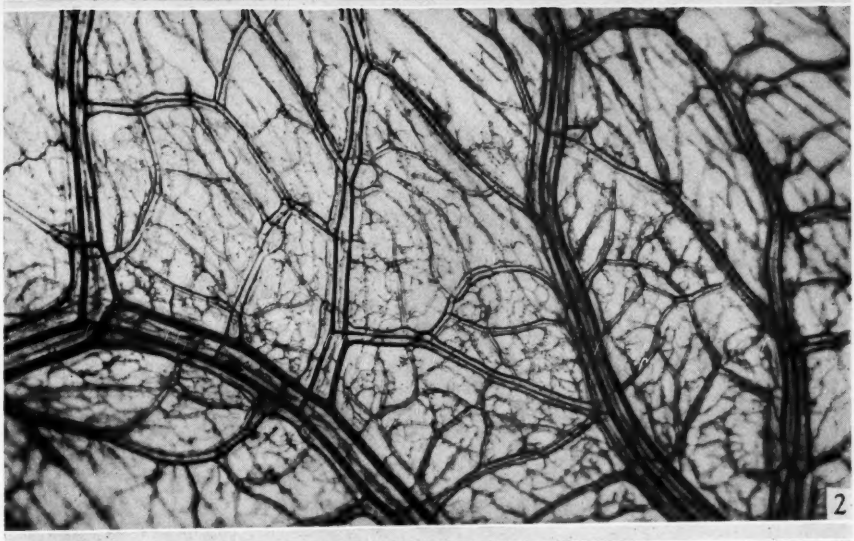
PLATE 2

Fig. 4. A projection drawing of intra-tendinous blood vessels injected with Indian ink. Note the longitudinal arrangement of arteries and venae comitantes with transverse anastomoses. These vessels lie in the interfascicular tissue. (×20.)

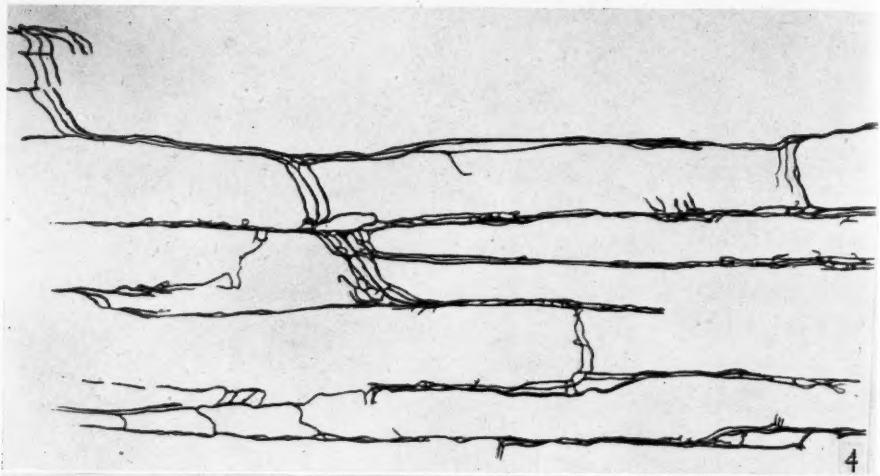
Fig. 5. A projection drawing of intra-tendinous lymphatics containing Indian ink, to the same scale as fig. 4. Contrast the size and shape of the vessels with those in fig. 4. The distribution of the vessels is seen on section to be perivascular. (×20.)

Fig. 6. A diagrammatic reconstruction of a tendon fascicle to show the longitudinal and transverse arterial channels and their venae comitantes.





EDWARDS—The blood supply and lymphatic drainage of tendons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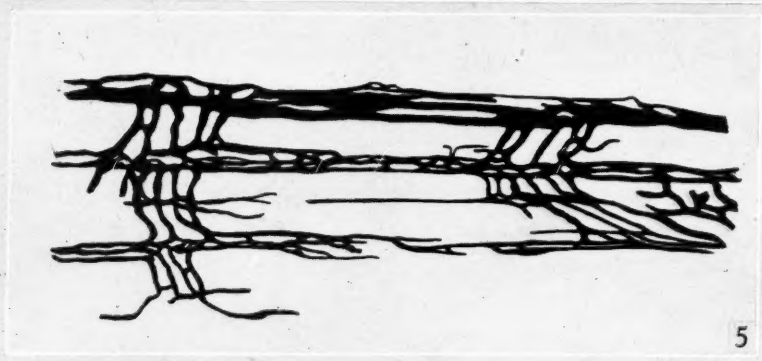
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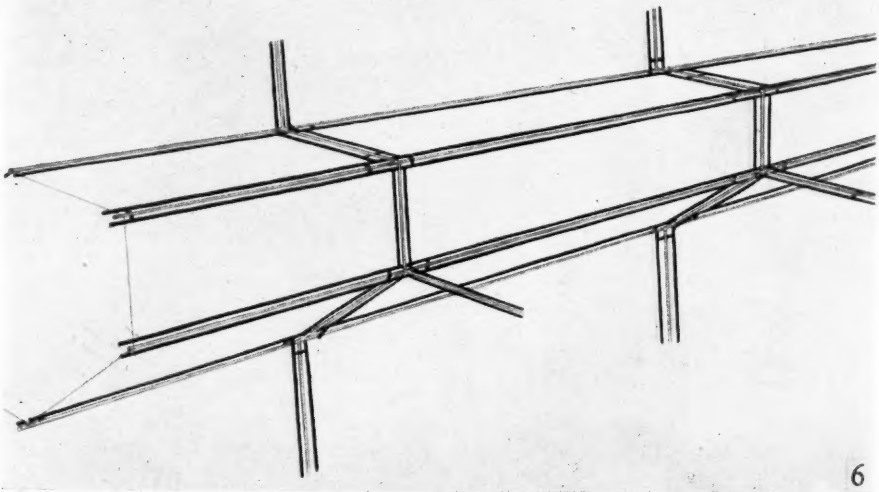
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THE VISUAL PATHS IN A CASE OF UNILATERAL ANOPHTHALMIA WITH SPECIAL REFERENCE TO THE PROBLEM OF CROSSED AND UNCROSSED VISUAL FIBRES

By T. ROGALSKI, *Department of Anatomy, Polish School of Medicine, University of Edinburgh*

Though the endings in the lateral geniculate bodies of crossed and uncrossed visual fibres are known with almost complete certainty and have been accurately plotted by Minkowski (1920, 1939), Clark (1932, 1942) and others, there is still some doubt (*a*) as to the proportion of crossed to uncrossed fibres in the optic tracts, (*b*) on what the proportion depends, and (*c*) as to the topographical arrangement of their endings in the area striata of the cortex. In this paper I would like to call attention to my own observations on the brain of a rare case of unilateral anophthalmia because, in comparison with the findings in normal brains, they may perhaps provide some contribution to the solution of those problems.

MATERIAL AND METHODS

The material consisted of three young brains: one of an 11-week-old child with complete congenital unilateral anophthalmia (Rogalski, 1944) and two normal brains as controls—one of a newborn, and one of a 2-day-old child. Selected portions of all these brains were embedded in celloidin and cut in serial sections. The anophthalmic brain had already been fixed in Kaiserling's fluid before it came into my hands. This restricted the use of subsequent staining methods. It was, however, possible to use Loyez's method, which actually proved to be advantageous as it was possible by this method to stain both nerve cells and fibres in the same section. The two normal brains were fixed in 10% formol saline and stained with thionin-blue (lateral geniculate bodies and visual cortex) or by Loyez's method (chiasma). The investigations were carried out by means of sections as follows: (1) Horizontal of the chiasma; (2) Almost transverse of the optic tracts; (3) Coronal of the geniculate bodies; (4) Coronal of the optic radiation; (5) Coronal of the occipital lobes—1, 2 and 3 had a thickness of 30 μ, 4 and 5 a thickness of 40 μ.

OBSERVATIONS

The left retina and optic nerve were completely absent, and a true chiasma was therefore absent also, but the right optic nerve divided in the chiasmatic region into a larger left optic tract and a much smaller right.

Chiasma and optic tracts. On macroscopic examination it is seen that many more fibres of the right optic nerve pass into the left tract than into the right, but on examining sections of the tracts it is found that the nerve fibres in each are evenly distributed so that no area of the section is devoid of fibres (Pl. 1, figs. 1, 2). This suggests that in the normal tract the fibres are similarly arranged and that crossed and uncrossed fibres are intermingled and not collected each in separate bundles, which is in agreement with the findings of Clark (1941) for the monkey, but opposed to those of Balado & Franke (1937) for man.

Since the sections through the tracts were not quite transverse it was difficult to count the fibres, but enlarged drawings of sections were made on millimetre paper, and it was thus possible to form an accurate estimate of the size of each tract.

At the magnification employed, the area of the left tract was 96 sq.mm. and of the right 48 sq.mm. Thus 66·66% of the total area of the two sections is constituted by the left tract, and 33·33% by the right. The actual area of the cross sections without magnification was 3·25 sq.mm. and 1·625 sq.mm. for left and right tracts respectively. Text-fig. 1, showing slightly enlarged transverse sections of the two tracts superimposed, gives a clear idea of their respective sizes.

As has been pointed out, no part of the section is devoid of fibres, but they are evenly scattered throughout the section. The surface area of the section may thus be taken to represent the proportion of crossed to uncrossed fibres in the two tracts, and this admittedly rough method gives the result that the left tract carries two-thirds and the right one-third of all the fibres of the right optic nerve—in other words the proportion of crossed to uncrossed fibres is 2:1.

It is true that some of the fibres in the tract run to the pretectal and tectal regions, but this would probably alter the ratio only slightly as the assumption is (Minkowski, 1920) that the percentage of uncrossed fibres running to these regions is small.

I have found further confirmation of the ratio of 2:1, by computing the areas of the temporal and nasal parts of the visual field served by crossed and uncrossed fibres respectively. For this purpose a chart of the uniocular visual field in man (from

H. M. Traquair's book on Clinical Perimetry, 1942, fig. 5), was cut by a vertical line through the macula (Text-fig. 2).

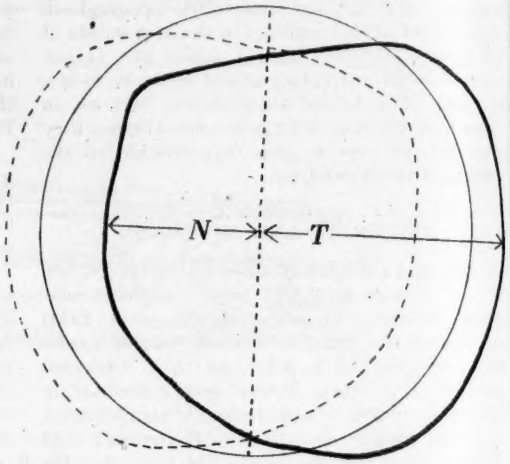
A calculation was now made of the areas of the temporal and nasal parts of the field. The areas are unequal: 66·9 % of the whole visual field corresponds to the temporal part, served by crossed fibres (inclusive of the lateral crescent), and 33·1 % to the nasal part, served by uncrossed fibres. Thus almost the same ratio is obtained; and this correspondence of the percentages (optic tracts and visual field) is obvious and striking. The correctness of the view that approximately half of the fibres in each optic tract are crossed and half uncrossed

L = 96 sq. mm. = 66·66% = 2/3

R = 48 sq. mm. = 33·33% = 1/3

Optic tracts

Text-fig. 1. Transverse sections of optic tracts, superimposed on each other.



T = 1982 sq. mm. = 66·9%

N = 982 sq. mm. = 33·1%

Text-fig. 2. Visual field of Man. *N*, nasal part; *T*, temporal part.

(Clark, 1943, p. 919) seems therefore rather doubtful.

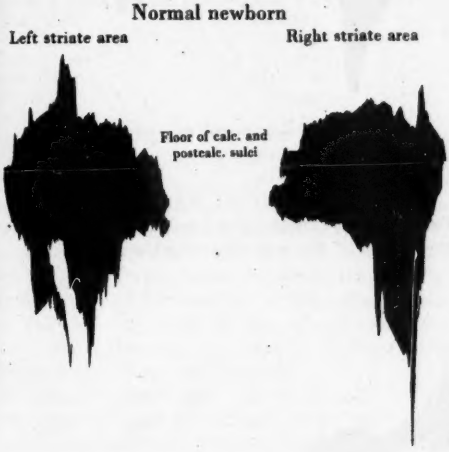
An indication that measurement of the sectional area of the optic tracts is of value in estimating the proportion of crossed and uncrossed fibres is to be found in the report of the case of a dog, examined by Rogalski & Rymaszewski (1945), which showed left-sided ocular cystic degeneration with complete destruction of the left optic nerve; the average ratio of crossed to uncrossed fibres was 4·5 : 1 (direct computation of fibres). Transverse section of the left tract measured 2·8 sq.mm., of the right, 0·6 sq.mm., the ratio being much the same.

Lateral geniculate bodies. Even normal geniculate bodies in man (Pl. 2, figs. 7, 8) do not show cell lamination as clearly as do those of the macaque, but resemble more those of the orang and chimpanzee. In the present anophthalmic specimen it is almost impossible to recognize any cell grouping, particularly on the right (Pl. 2, figs. 9, 10). On the left side it is just possible to make out three groups of cells, a lower layer of large cells covering the myelinated fibre layer, a middle layer of medium-sized cells, and an upper arched layer of medium-sized cells, but no definite laminae or cell-free areas can be made out. On the right side conditions are even more abnormal; cells and fibres are less numerous and the sections have thus a lighter colour; cells are scattered in clumps and dispersed in a disordered manner, but an indefinite collection of large cells can be seen in the lower part of the nucleus not distinctly separated from a collection of middle-sized cells above.

Study of serial sections shows that on the left side cells are more numerous in the anterior third, and in the central and medial parts of the middle third than in the lateral part of the middle third. The posterior third shows a well-defined lamina of large cells and a central group of medium-sized cells. On the right side cells in the anterior third and medial part of the middle third are less numerous than on the left.

Visual cortex. In the normal brains coronal sections through the calcarine and post-calcarine

sulci show the usual arrangement of the area striata, with lamina IV divided into three sublaminae, *a*, *b* and *c*, which is seen also in the particular part of the anophthalmic brain shown in (Pl. 1, fig. 6). When the same regions of the anophthalmic brain are examined a unistriatal instead of a tristriatal pattern is found, particularly round the deepest part of the sulci on the right side, and less markedly on the left (Pl. 1, figs. 4, 5, 6). The question then arises whether these unistriatal areas are really undifferentiated parts of the true area striata, or whether they are parts of the area parastriata that have become dislocated owing to disturbed development.

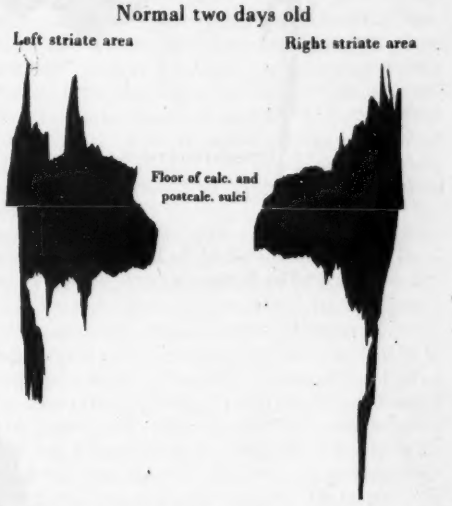


Text-fig. 3. Diagram of the striatal area in normal brain of new-born child. The horizontal line through the middle of the striatal area corresponds to the bottom of the calcarine and post-calcarine sulcus.

A careful study of these unistriatal areas under high magnification demonstrates that these areas are true striatal areas, and that they do not present the characteristics of the parastriatal area, but, except for the failure of any division of lamina IV into three sublaminae, they possess all the features of striatal cortex (visuo-sensory area).

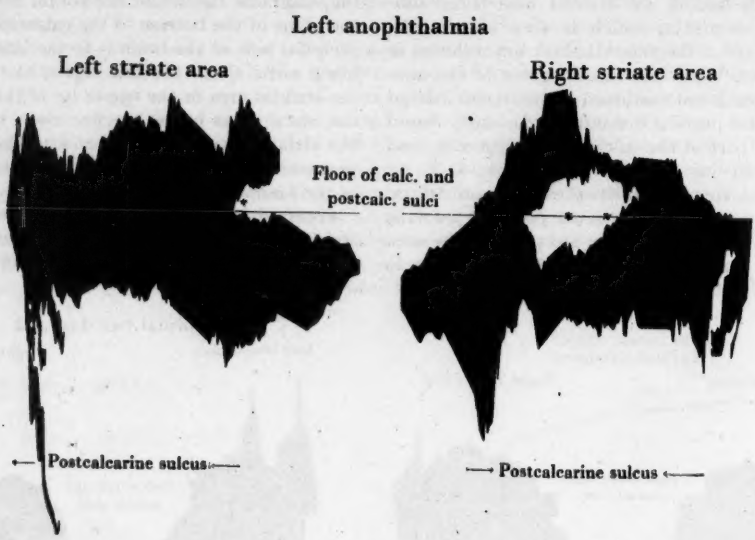
In order to demonstrate the extent of the unistriatal area relative to the tristriatal, a chart was prepared from drawings of every fifth section of the occipital lobes of all three brains, projected at a magnification of $\times 10$ on to millimetre paper. In the diagrams the white horizontal line represents the plane of the bottom of the calcarine sulcus; the occipital pole of the brain is to the lateral side; the black areas above the line represent the extent of the striatal area in the upper lip of the sulcus, and the black areas below the line show the extent of the striatal area in the lower lip. The tongue-like processes represent the extension on to the surface of the hemisphere (Text-figs. 3, 4, 5).

Comparing the normal with the anophthalmic specimen it is seen that in the diagrams of the latter there are areas in the anterior part of the left side

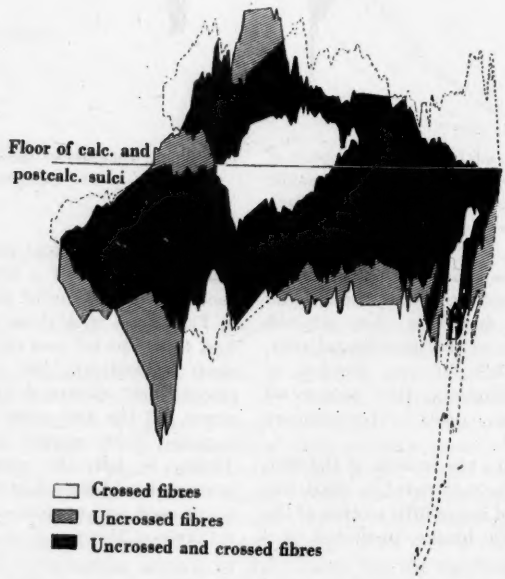


Text-fig. 4. Diagram of striatal area in normal brain of a 2-day-old child.

and in the middle and posterior parts of the right that are devoid of a tristriatal pattern, and are therefore left white (cf. places marked by asterisks in Text-fig. 5 with those noted by arrows in Pl. 1, figs. 4–6). The left and right sides of Text-fig. 5 thus show respectively the presumed termination of crossed and uncrossed fibres from the right optic nerve. If the two parts of Text-fig. 5 are superimposed there appear (Text-fig. 6) three areas. These, it may be presumed, were the brain normal, would represent the areas of termination of (*a*) crossed and uncrossed fibres, (*b*) uncrossed, and (*c*) crossed fibres.



Text-fig. 5. Diagram of striatal area in the brain of the anophthalmic child. The distance between the arrows indicates the length of the postcalcarine sulcus.



Text-fig. 6. Left and right striatal areas of Text-fig. 5, superimposed on one another.

DISCUSSION

It is of interest to notice that the evidence seems to point to the even distribution of crossed and uncrossed fibres in the optic tracts, and to their proportions as 2:1. Table 1 shows the estimates that have been arrived at by various workers in the case of man and other mammals:

Table 1

Author	Species	Ratio of crossed to uncrossed fibres
Nichterlein & Goldby (1944)	Sheep	9:1
Dexler (1897)	Horse	8:1
Rogalski & Rymaszewski (1945)	Dog	4·5:1
Bodian (1937)	Opossum	4:1
Packer (1941)	Phalanger	3:1
Jefferson (1940)	Ferret	3:1
Sântha (1933)	Man	3:2
Clark (1942)	Macaca	3:2
Cajal (1899)	Man	2:1
Rogalski (present article)	Man	2:1
Clark (1943)	Man	1:1

Consideration of this Table shows that there is a progressive diminution of the number of crossed to uncrossed fibres in the tracts and this seems to be associated with the degree of projection of the external nose and the recession of the eyeball in the face. The different ratios of crossed and uncrossed fibres in the Table does not seem to depend on the taxonomic position of the animals.

Tschermak (1902, 1927, 1939), Dubar (1924), Rochon-Duvigneaud (1922) and Pisă (1939) have shown that a binocular field with complete decussation of optic fibres exists in lower vertebrates also.

The whole arrangement of motor and sensory cortical areas and their association with the visual fibres is of great interest in this connexion (cf. Dixon, 1918).

With regard to the lack of cellular definition and lamination in the lateral geniculate body, especially on the right side, it is important to notice that the abnormal eye condition of this case must have begun at about the 5 mm. stage (Rogalski, 1944), but that the primordium of the lateral geniculate body is hardly recognizable even at the 16 mm. stage. One is naturally led to speculate as to how far the conditions found in the lateral geniculate body are due to an inherent deficient development of the geniculate body itself, and how far they are due to an absence of the stimulus of afferent fibres from the optic tract. It has been shown above that the right body is the more affected, and it is the right tract that is the smaller. Furthermore, the geniculo-cortical fibres passing from the right body are less dense than on the left. Again, the absence of the subdivision of cortical lamina IV, into the normal sublaminae must be associated with a developmental deficiency. If, as in Text-fig. 6, the spread-out visual cortex of right and left sides be superimposed, there will be three different areas that can be considered, as described above, and these may each be regarded as the areas for the endings of the particular variety of fibres, so that the smallest area would be for the uncrossed fibres, the middle-sized for the crossed, and the largest an area where crossed and uncrossed are mingled. This is at variance with the current opinion, based mainly on the experiments of Poliak (1932, 1933), and Clark (1941) on the macaca brain. In all these experiments, however, even the smallest lesions were situated in the antero-lateral part of the striatal cortex, the part which corresponds in Man more or less to the polar region near the end of the postcalcarine sulcus. This area contains the macular centre, and the diagram of the anophthalmic brain shows that this subarea is destined to receive both kinds of fibres. For the same reason it becomes clear that a lesion in that part (the macular region) must have involved all six laminae in the lateral geniculate body.

The result of investigations of the visual cortex in cases of anophthalmia or of blindness have hitherto been mostly contradictory. Panizza (1855), Leonowa (1893, 1896), Cramer (1898), Bolton (1900), Gallemaerts (1902), Hanke (1904), Henschen (1910), Monakow (1914) and Schroeder (1929) all found in such cases defects in the lamina granularis interna of the visual cortex. Tsang (1937) described thinned striatal cortex and withering of the cellular elements in rats, and Chase (1945) showed thinned lamina II, III and VI, and the two first less separated from the narrowed lamina IV in mice. These findings would appear to point to the interdependence of the three optic neurones. On the other hand, Triepel (1921), Woollard (1926), Chasan (1927), Cosmettatos (1931), Clark (1932), Sântha (1933), and Hechst (1933) affirm that no visible changes have been seen in the visual cortex. The latter observations point rather to the independence of the separate neurones in their development and behaviour.

Among the observations quoted the most interesting are those which deal with a *unilateral* experimental lesion, or with recent or long-standing *unilateral* blindness. Three authors found cortical changes (Henschen, 1910; Tsang, 1937; Schroeder, 1929), and three did not observe any changes (Clark, 1932; Sântha, 1933; Hechst, 1933). But many of these observations, positive as well as negative, should be accepted with reserve because none of the authors mentions that the investigations were made on continuous serial sections throughout the striatal area, and without the fulfilment of this condition no conclusions can safely be reached. Moreover, another point, to which Schroeder called

attention, must be taken into consideration. He examined a case of complete left blindness lasting 50 years. So long as he used slight enlargement he could not find any difference between the two sublaminae *a* and *c*; both showed a dense arrangement of cells. But higher magnification proved that many of these cells were not nerve cells at all, but were neuroglial. When he then computed the number of both types of cells he concluded that an obvious nerve-cell atrophy existed in the heterolateral lamina IV*c* and in the homolateral lamina IV*a*.

The remarks of Chasan (1927) and Sântha (1933) should be mentioned too. Although they did not find any evidence of changes in the striatal area, they considered that one should be chary of drawing inferences from the absence of changes in the cortex because here one is dealing with transneuronal atrophy of the third neurone. And Tsang (1937, p. 15) concluded that 'time was a decisive factor in inducing degeneration of the visual centres'.

In general, until now, there exist three different views regarding the endings of crossed and uncrossed fibres in the visual cortex: (1) both types are intermingled in both sublaminae *a* and *c* of lamina IV everywhere; this is the opinion most commonly held; (2) the crossed end in IV*c*, and the uncrossed in IV*a* (Kleist, 1926; Gurewitsch, Bychowsky & Uranowsky, 1929); (3) both types end in IV*c* (Bárány, 1925). I may mention in passing the old view of Wilbrand & Saenger (1904), who suggested that the impulses from each eye were conducted to separate parts of the visual cortex where, like a chessboard, the fields of the corresponding points of both retinae lay one beside the other.

From the observations made on this case of complete unilateral anophthalmia it seems possible that there may exist in the striatal area three subareas, the positions of which have been already stated. However, as this suggestion is based on a single case only, the important problem of the cortical endings of homo- and heterolateral visual fibres must be considered as still in need of further careful investigation.

SUMMARY

1. Observations have been made on the centres of three young brains (two normal and one with complete left anophthalmia).

2. The proportion of crossed and uncrossed optic fibres has been indirectly estimated as 2:1, which agrees with the ratio of the relative dimensions of the temporal and nasal parts of the visual field in each eye.

3. Diagrams of the striatal areas in the three brains were made with the aid of millimetre-scale paper. They show clearly the form and extent of the visuo-sensory cortex, when laid flat.

4. The evidence from the anophthalmic brain suggests that there may be three topographical subareas in the visuo-sensory cortex: (*a*) a large one destined for the endings of crossed and uncrossed fibres; (*b*) a medium for crossed fibres only; and (*c*) a small for uncrossed only.

I offer my thanks to Prof. A. M. Drennan for the use of the normal brains and to Prof. J. C. Brash and Dr R. F. Ogilvie for placing at my disposal the anophthalmic brain. My thanks are due also to Mr Borthwick of the Department of Anatomy, University of Edinburgh, for his skill in preparing the photomicrographs. I acknowledge also my gratitude to Dr O. Rymaszewski, Demonstrator in the Department of Anatomy of the Polish School of Medicine, for much technical assistance.

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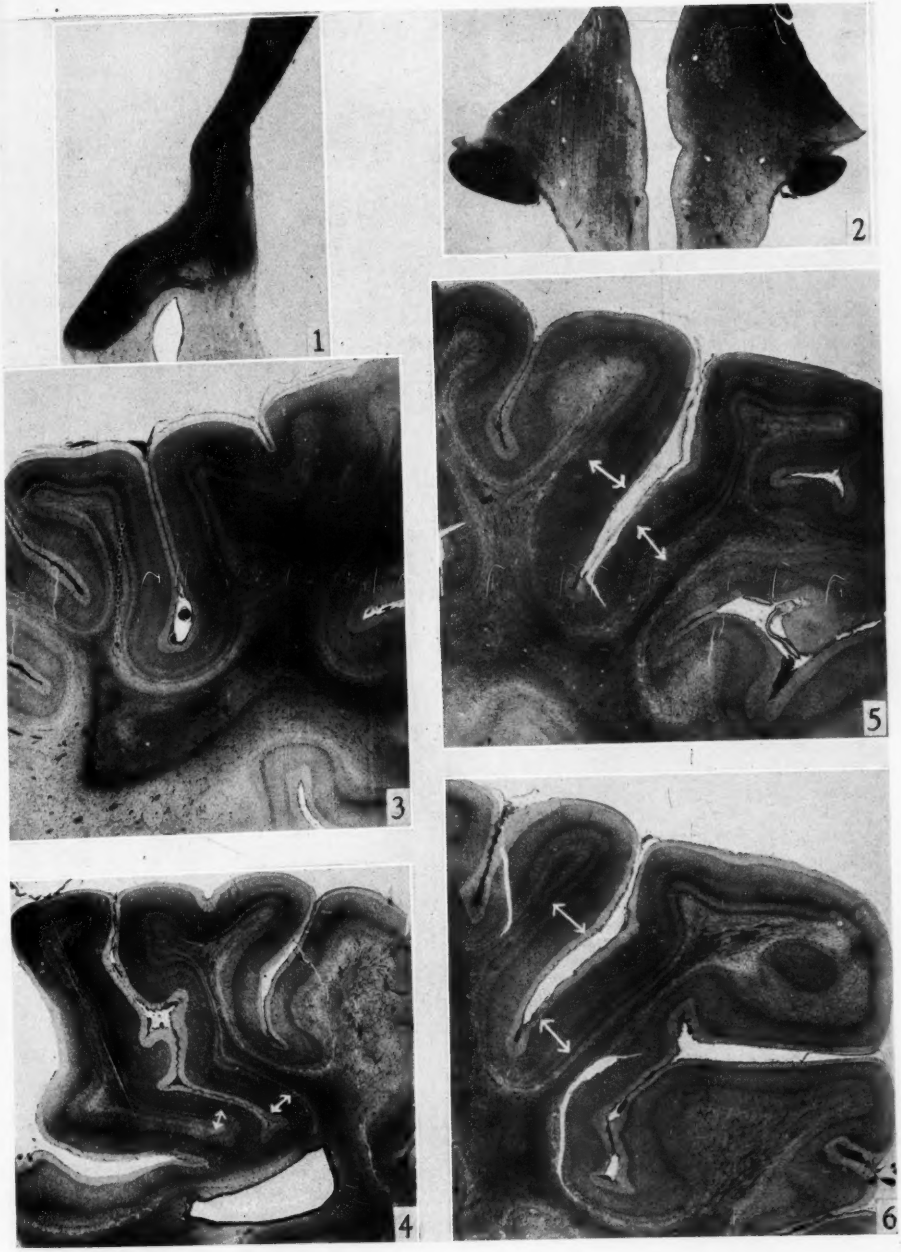
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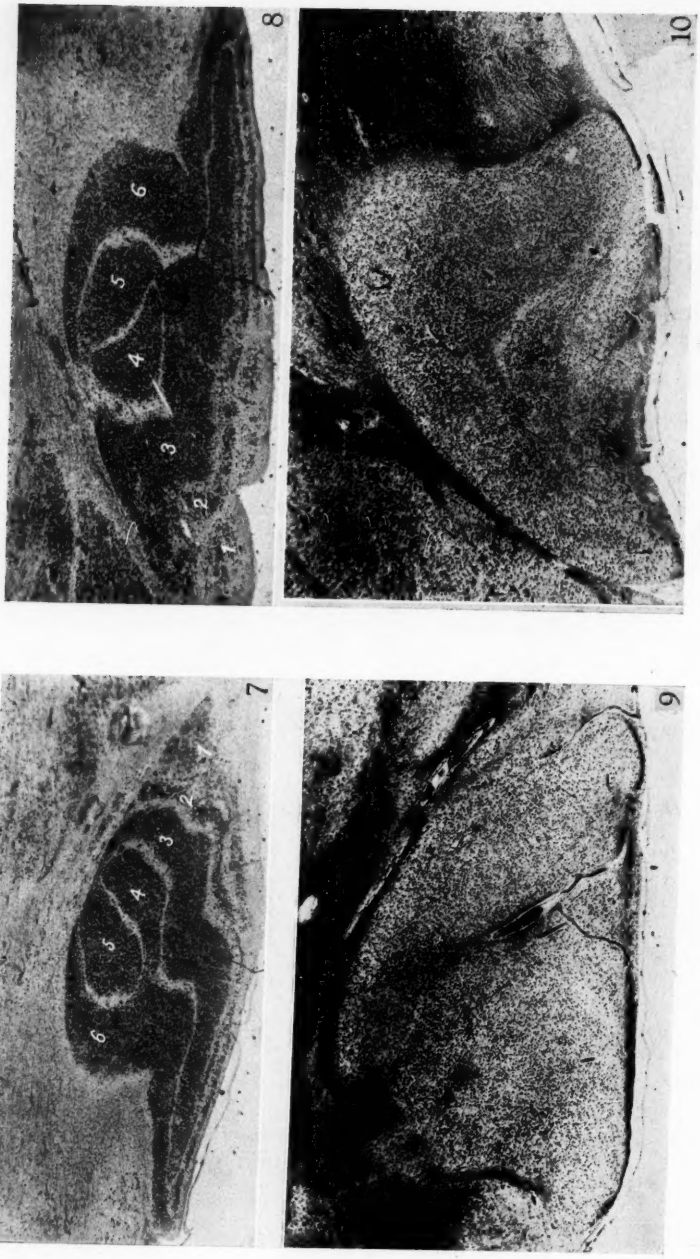

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EXPLANATION OF PLATES

PLATE 1

The arrows marked on figs. 12–14 define the parts devoid of tristriatal pattern.

All these figures (1–6) relate to the anophthalmic brain.

Fig. 1. Division of right optic nerve into homo- and heterolateral fibres. ×5.
Fig. 2. Coronal section through both optic tracts. ×5·5.
Fig. 3. Coronal section through middle part of left postcalcarine sulcus. ×4·75.
Fig. 4. Coronal section through frontal part of left postcalcarine sulcus. ×4·75.
Fig. 5. Coronal section through middle part of right postcalcarine sulcus. ×5·25.
Fig. 6. Coronal section 12 mm. caudal to fig. 5. ×5·25.

PLATE 2

Fig. 7. Coronal section through middle part of right lateral geniculate body of normal 2-day-old child. ×25.
Fig. 8. Similar section on the left side. ×25.
Fig. 9. Similar section through right lateral geniculate body of anophthalmic brain. ×25.
Fig. 10. Similar section on the left side. ×25.

THE EARLY DEVELOPMENT OF THE CORPUS LUTEUM IN THE MARE

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INTRODUCTION

Although the macroscopic changes occurring in the ovary of the mare during the oestrous cycle have been fully described, there is relatively little known of the associated microscopic changes or of the formation of the corpus luteum. Seaborn (1925) followed the oestrous cycle and described the changes in the ovary in nineteen mares, and Kupfer (1928) reported on the macroscopic changes found in 360 South African donkeys and horses. Day (1940) determined the length of oestrus, dioestrus, and the time of ovulation in eight pony mares. Hammond & Wodzicki (1941) corroborated Day's findings and reviewed the gross changes that occurred in the reproductive organs of six mares. The above investigators mention only the macroscopic changes in the appearance of the developing corpus luteum. Seaborn & Champy (1923) give a brief description of the development of the corpus luteum and say: 'les cellules à lutéine sont ici d'origine thécale, très sûrement.' Cole, Howell & Hart (1931) divide the period of development of the corpus luteum of pregnancy into three stages, and remark (p. 205) on its 'lace-like' structure in the youngest stage. Haller (1939) describes the retrogression of the corpus luteum during the later part of pregnancy.

The present work was undertaken to investigate the histology of the early corpus luteum in a series of animals in which the ages of the corpora lutea were fairly accurately known. In some of these animals ova had been recovered from the tube or uterus.

MATERIALS AND METHODS

Specimens of corpora lutea obtained at periods after ovulation varying from 2 hr. to 17 days were employed. They are divided into two series: (1) from 18 hr. to 17 days, (2) from 2 to 21 hr. In the first series (Table 1) thoroughbreds A and B and P (1–8) were specimens from animals used by Hamilton & Day (1945) to whom I am greatly indebted. The remainder of the series were obtained by Mr Burkhardt and myself from other animals, following the method of Hamilton & Day for estimating the time of ovulation. Only the animals in which the time of ovulation was fairly accurately determined are included in the table.

In order to obtain the very earliest specimens possible, recourse was had to artificial rupture of the follicles in the manner described by Day (1939*a*) for the puncture of ovarian cysts or follicles. Briefly the procedure involves the passage of a trochar through the vaginal wall into the follicle, the ovary being held by the other hand in the rectum. Success in the operation produced a flow of as much as 20–30 c.c. of follicular fluid down the trochar. The ovaries were always palpated beforehand until an enlarging follicle was felt to be 3–4 cm. in diameter. The mare was then killed a definite time after puncture, and the ovary placed in fixative after being sliced in two. Seven mares (Table 2) were treated in this way.

Pieces of the developing corpora lutea, or of punctured follicles, were then sectioned at 5–10 μ and stained variously with Delafield's haematoxylin and eosin, Heidenhain's haematoxylin, Mallory's triple stain, Masson's stain, and with osmic acid. Sections were also stained by Robb Smith's modification of Foot's method for reticulum and collagen.

FOLLICULAR DEVELOPMENT

A number of small follicles (1 cm.) appear in the ovary as early as 14 days before oestrus commences (Day, 1939*a*; Hammond & Wodzicki, 1941). They tend to be arranged in rows tangential to the hilum (ovulation fossa) of the ovary (Kupfer, 1928). Two or three days prior to oestrus a few of these small follicles enlarge more than the others, and during the early days of oestrus one of these latter enlarges rapidly to a diameter of 3–4 cm., due to the secretion of secondary liquor. Occasionally two very large follicles may be present in one ovary. Kupfer's observation (1928) that greatest growth occurs at the poles of the ovary is corroborated in the present series.

In the smaller immature follicles (Pl. 1, fig. 1) the granulosa cells are tightly packed and have intensely staining nuclei. The theca interna cells are seen, two or three layers thick, circumferentially arranged deep to the granulosa cells. A well-marked limiting membrane can be seen with Mallory's staining between the granulosa and theca interna. The thecal cells have a flattened appearance, little cytoplasm and no osmiphile granules. Their nuclei are elongated and show a few mitotic figures.

In the mature follicles (Pl. 1, fig. 2) the granulosa cells have increased in size and those nearer the cavity of the follicle contain more cytoplasm whilst their nuclei have become larger and spherical. The granulosa cells in contact with the limiting mem-

brane have become columnar in appearance; their nuclei are at the base of the cell near the membrane and are elongated, pointing to the centre of the follicle. The theca interna shows an increase in the number of cells, though very few mitotic figures can be seen at this stage. The cells show an increased amount of cytoplasm and have a more granular appearance. They are no longer flattened and arranged circumferentially, but are becoming polyhedral in shape and are distributed in various planes. Their nuclei also are larger, more spherical and vesiculated. Increase in the number of thecal capillaries is also marked. With the sudden marked enlargement of the follicle during oestrus the two follicular layers become relatively thinner and more stretched and the theca interna cells may not always show the pronounced change in shape from spindle to polyhedral type, but there are always an increased amount of cytoplasm and changes in the nuclei. No bodies of Call and Exner have been observed in any follicle.

These changes in the theca interna are similar to those described by Stafford, Collins & Mossman (1942) in the guinea-pig, Warbritton (1934) in the ewe, Dawson & Friedgood (1940) and Dawson (1941) in the cat, and Strassmann (1923) and Shaw (1925) in man. There is no doubt that this proliferation and hypertrophy in the theca interna of the mare prior to ovulation qualifies for the description of a 'thecal gland' as suggested by Mossman (1937) in the pocket gopher.

ATRESIA

The signs of atresia are apparent in the smaller follicles of 1–2 cm. even before ovulation from the largest follicle has occurred. The nuclei of the granulosa cells become coalesced into a chromatic mass and gradually become rounded. Chromatolysis now takes place and the granulosa cells take on an appearance very like that of a leucocyte. The theca interna cells never become polyhedral in shape but remain in an undeveloped state with none of the changes of a progressive thecal gland. The outer cells show evidence of invasion by theca externa cells and dense collagenous fibres are laid down round the degenerating theca interna cells. These changes are most clearly seen in follicles adjacent to a forming corpus luteum. The ultimate fate of the atretic follicular cells has not been determined.

DEVELOPMENT OF THE CORPUS LUTEUM

Ovulation in the mare is spontaneous and occurs on the 6th day of oestrus about 24 hr. before the end of oestrus (Mirskaia, 1935; Day, 1939*a*, *b*; Hammond & Wodzicki, 1941). The ages of the present series of specimens are all dated from estimations of ovulation by rectal palpation and the times are probably accurate to within ±6 hr. In ten of the series ova were recovered and the cleavage stages can be compared with the corresponding ages of the corpus luteum (Table 1).

Table 1

Animal	Date killed	Age of corpus luteum (hr.)	Stage of development of ovum	Insemination	Dosage of gonan in i.u.
D45	6. vi. 45	18– 24	—	—	—
Thoroughbred C	22. xi. 45	18– 24	—	—	—
P5	8. viii. 44	18– 24	2-cell	+	—
P1	22. v. 44	24– 30	Unsegmented	+	750
P2	29. vi. 44	27– 33	3-cell	+	1000
P7	3. vi. 44	30– 36	4-cell	+	1000
D40	18. iii. 45	36– 44	—	—	—
Thoroughbred A	16. ix. 44	44– 50	Degenerating	+	—
P4	19. v. 44	50– 56	5-cell	+	1000
Thoroughbred B	16. ix. 44	68– 74	Degenerating	+	—
P8	5. vi. 44	78– 84	Unsegmented	+	1000
P6	5. vi. 44	94–100	Morula	+	1000
DX	18. iii. 45	116–122	—	—	—
P3	12. ix. 44	144–168	In uterus	+	1500
D7A	18. iii. 45	240–280	—	—	—
D17	18. iii. 45	390–410	—	—	—

In the youngest specimens (18–24 hr.) the follicle has collapsed causing folding of the wall. A central cavity contains a little secondary liquor folliculi: in all early specimens there is haemorrhage into the lumen of this cavity. The arrangement of the lutealizing granulosa cells shows that two types of developing corpora lutea can be distinguished as Pearson & Enders (1943) found in the fox.

In the first type, the commonest in this series, the granulosa presents the open 'lace-like' form described by Evans & Cole (1931) in the dog, and observed by Cole *et al.* (1931) in the mare. The cells are elongated and stretch out in irregular columns into the lumen of the collapsed follicle (Pl. 1, fig. 5).

These authors give no explanation of this reticulated appearance, but in the mare the explanation seems to be that the haemorrhage from the ruptured thecal capillaries causes a disintegration of the granulosa so that the corpuscles can pass into the centre of the collapsed follicle.

The second type shows a more compact arrangement of the lutealizing granulosa with the haemorrhage into the follicle occurring at isolated points and without disrupting the granulosa (Pl. 1, fig. 3). In both types the granulosa cells are undergoing lutealization in the usual manner, their cytoplasm gradually increases in amount and becomes granular, the osmic acid staining elements are increased in number and the nuclei enlarge and become vesiculated. There is no evidence of mitoses in the granulosa cells at this stage.

In both types the theca interna cells are seen sparsely scattered at the outer edge of the granulosa as a few isolated cells occasionally showing vacuolation. The theca interna cells are also carried out to the ends of the folds of the mural granulosa. They are no longer separated from the granulosa by the limiting membrane. The separation of the luteal cells from the antrum by a connective tissue layer described by Cole *et al.* (1931) and said to be due to an ingrowth of theca externa cells was not observed in any of the early specimens.

A section of the early developing corpus luteum stained by Robb Smith's modification of Foot's method shows the trabeculae of the theca externa heavily impregnated with silver. There is only a slight deposition of silver in and around the luteal cells (Pl. 1, fig. 4).

The next stages (30–48 hr.) show an increase in the size of the luteal cells and the characteristics of lutealization are more marked. The follicle is not yet completely filled by the growing corpus luteum and a dark central area can be seen on the cut surface. The theca interna cells have now lost all characteristics of a thecal gland and have begun to invade the mural granulosa. They can be seen interspersed among the luteal cells most markedly at the apices of folds. These folds now take on the appearance of trabeculae and the striking trabeculation of the mare's corpus luteum distinguishes it from the corresponding structure in other animals. The trabeculae consist of a central core of theca externa cells with the degenerating theca interna cells invading the now lutealizing granulosa cells on all sides. At the same time the vascularization of the corpus luteum begins from the thecal capillaries and endothelial cells can be seen insinuating themselves between the luteal cells. The trabeculae are also extensively vascularized and large vessels may be seen at the bases of most of them.

By the 4th day (Pl. 1, fig. 6) the corpus luteum nearly fills the cavity of the collapsed follicle and on the 6th day (Pl. 1, fig. 7) the opposing walls are separated only by a small central plug of degenerating corpuscles surrounded by fibrin, with a few thecal cells invading the matrix. The whole corpus luteum is now well vascularized and the thecal cells are evenly distributed among the very large luteal cells. The trabeculae diminish in size and become merely a support for the vessels passing into the corpus luteum. The theca externa cells begin to pass into the corpus luteum lying between the luteal cells where they can be differentiated from the theca interna cells by their flattened spindle shape, as opposed to the rounded and smaller cells of the theca interna.

At 14 days the corpus luteum (Pl. 2, fig. 8) has reached its maximum size and fills the collapsed follicular cavity, though it is smaller in diameter than the original follicle. From now on the corpus luteum gradually diminishes in size. For the later changes in pregnancy some account will be found in Cole *et al.* (1931) and Haller (1939). The luteal cells, now very large, show a peripheral vacuolation: their nuclei have a granular but homogeneous area about them as described by various authorities in other animals (see Corner (1932) for a review).

The fully developed corpus luteum stained by Robb Smith's modification of Foot's method shows the trabeculae heavily impregnated with silver, and there is a deposition of silver in the form of a fine network about the luteal cells. The position of the theca interna cells is marked by heavily impregnated areas, agreeing with the observations of Solomons & Gatenby (1924) on the corpus luteum of the pig and other animals. These heavily impregnated areas are interspersed in the fine network between the luteal cells (Pl. 2, fig. 9).

ARTIFICIALLY RUPTURED FOLLICLES

In order to fill the obvious gap in the above series from ovulation time to the earliest normally developed corpus luteum a further series of mares in which the follicle had been ruptured, in each case as near ovulation as possible, was employed, the mare being killed after a definite interval. Of seven mares so treated, five showed histological changes in the granulosa or theca interna (Table 2), whilst in one there was no change and in one an ovarian cyst was punctured in mistake for a follicle.

The first animal, killed 2 hr. after puncture, showed a collapsed follicle with the granulosa intact and the theca interna showing early changes of a developing thecal gland (Pl. 2, fig. 10).

In the second animal, killed 5 hr. after puncture, the granulosa cells are still unchanged, but there is marked enlargement of the theca interna. The cells have increased in number and are polygonal in shape. In one area of the follicle (Pl. 2, fig. 11) the

Table 2. *Artificially ruptured follicles*

Animal	Date killed	Puncture-killing interval (hr.)	Remarks
Pp3	9. vii. 45	2	No change
Pp4	16. vi. 45	5	Granulosa unchanged, theca interna markedly increased
Pp5	2. vii. 45	8	Granulosa unchanged, great hypertrophy of theca interna
Pp1	21. iii. 45	10	Granulosa unchanged, great hypertrophy of theca interna
Pp2	31. iii. 45	18	Cystic
Pp18	22. xi. 45	18	Granulosa shows signs of lutealization. Theca interna diminishing
Pp6	2. vii. 45	21	Granulosa lutealizing. Theca interna diminishing

thecal cells show a great increase in size and changes almost simulating those seen in lutealizing granulosa cells. The granulosa cells are raised from the follicle wall by the hypertrophying thecal cells, and in many parts of the follicle have broken away from the wall. The limiting membrane is stretched by the thecal hypertrophy and in most places has been ruptured. The thecal vessels have enlarged all round the collapsed follicle and have ruptured in a number of places to give rise to an intrafollicular haemorrhage which has broken the granulosa cells into irregularly spaced clumps. The hypertrophy of the thecal cells is most noticeable in areas immediately adjacent to large thecal vessels. There is little evidence of mitosis in the thecal cells, and the increased prominence of the zone is apparently mainly due to an enlargement of the cells rather than to an increase in their number.

In the third and fourth animals, killed 8 and 10 hr. after puncture of the follicle, the appearances are very striking (Pl. 2, figs. 12, 13). The collapsed follicle is invaded from all sides by tufts or protuberances of rapidly hypertrophying theca interna cells. The granulosa is thrown into folds by these tufts, but its cells show little evidence of lutealization. At the ends of the tufts the thecal cells are much hypertrophied, and a few mitoses can be seen. The nuclei are enlarged, spherical and vesiculated. The limiting membrane has been broken down at the end of the tuft and the thecal cells are invading the granulosa cells. The mural granulosa still shows little evidence of lutealization, but the thecal cells, though fewer in number, show the changes described above (Pl. 2, fig. 14). In many thecal cells the cytoplasm is definitely granular. The intrafollicular haemorrhage is not so marked as in a spontaneously ruptured follicle, but in one of these specimens not all the follicular fluid was removed.

In the sixth and seventh animals, killed 18 and 21 hr. after puncture, the same folded appearance of the follicular wall can be seen. There is, however, a more marked haemorrhage into the collapsed follicle than in the earlier specimens. The granulosa cells form a lace-work structure round the wall, with blood corpuscles separating them (Pl. 2, fig. 15). These cells begin to show evidence of lutealization: they are more rounded, their nuclei have become vesiculated and spherical, and their cytoplasm is gradually increasing in amount and becoming granular. The tufts of theca interna are now less marked and are invaded by theca externa cells at the base. The theca interna cells show a diminution in size from the last two specimens, and in some cells vacuolation can be seen. Many of the thecal cells are passing out among the outer layer of granulosa cells.

DISCUSSION

There is little doubt that there is an enlargement of the theca interna cells in the mature follicle of the mare which indicates some kind of cellular activity. These changes are similar to those described in other animals by Mossman (1937) and reviewed by Stafford *et al.* (1942). The presence of a thecal gland has now been demonstrated in a large number of animals, e.g. squirrel, guinea-pig, sheep, goat and man. In the rhesus monkey the theca interna cells are never very conspicuous, but at ovulation they are larger than the granulosa cells and contain a considerable amount of lipid material (Corner, 1945). In the mare also there is an increase in the size and number of the theca interna cells, but the most striking changes occur between 5 and 10 hr. after a follicle has been artificially ruptured just before the estimated time of ovulation. A large number of tufts of actively enlarging thecal cells grow out into the cavity of the collapsed follicle, and the changes in the cells simulate those of lutealization of the granulosa.

Strassmann (1941) has found that the theca interna cells proliferate also during the early growth of the follicle in the mare and by their eccentric development form a wedge-like 'theca interna cone' which points to the hilum of the ovary. He suggests this cone functions as a 'pathmaker for the ascent of the growing follicle to the ovarian surface'.

It has been postulated that this hypertrophy of the theca interna zone prior to ovulation marks an active secretory phase on the part of these cells and that the substance secreted may be oestrogenic in nature (Corner, 1938, for review; Stafford *et al.* 1942). Hisaw (1925) has shown that in the pocket gopher the injection of oestrogenic hormones causes a resorption of the symphysis pubis. Mossman (1937)

indicated that this resorption may be associated with the activity of the thecal gland which reaches its maximum growth in the pocket gopher when these changes in the symphysis pubis are found. Until such a hormone is isolated from the theca interna cells this evidence of thecal secretory activity must be presumptive.

The striking changes that occur in the theca interna between 5 and 10 hr. after a mature follicle has been artificially ruptured may be due merely to a release in pressure in the follicle. This is unlikely as the thecal cells are enlarged before puncture and the granulosa cells remain relatively unaffected for some hours after the thecal cells have shown their proliferation. Since this thecal activity is most marked in the early punctured follicles in the neighbourhood of dilated thecal vessels it is possible that the stimulant for this activity is blood borne. Allen, Danforth & Doisy (1939) suggest that small quantities of the lutealizing hormone secreted by the anterior pituitary may be present in the circulation prior to ovulation. The changes that occur in the theca interna after artificial rupture of the mature follicle do resemble the changes that are later to occur in the granulosa cells. Therefore it is possible that these changes in the theca interna are due to the effects of a lutealizing hormone acting on the theca interna cells first before reaching the granulosa cells.

A more probable explanation is that the thecal changes after rupture are due to the action of the follicular stimulating hormone of the anterior pituitary. This hormone is also blood borne and if the supposed function of the thecal gland is to produce an oestrogenic hormone, the thecal cells would quite likely be stimulated by this hormone. It has been remarked above that ovulation in the mare occurs 24 hr. before the end of oestrus as demonstrated by biological assessment. Therefore it is likely that there is a certain amount of this follicular stimulating hormone still acting on the follicle during this time and producing the marked changes in the theca interna. The release of pressure in the process of puncturing the follicle merely allows more freedom for the changes to occur.

Artificial puncture of the mature follicle has been performed in other animals, but in those animals that do not ovulate spontaneously copulation must precede the puncture (Regaud & Dubreuil, 1909). In the dog (Marshall & Runciman, 1914) and in the monkey (Allen *et al.* 1939) artificial rupture of the follicle is followed by lutealization of the granulosa. Westman (1934) has shown that puncture of mature ovarian follicles in the dog and removal of the unfertilized egg does not affect the subsequent development of the granulosa cells into luteal cells. The animals used in those experiments were killed between 2 and 5 days after the follicles had been punctured and no change in the theca interna was reported. Puncture of ovarian follicles and cysts in the mare has been a standard practice in treating irregularities of the oestrous cycle for some time past (Day, 1939*a*) and the operation results in the production of a corpus luteum in the ruptured follicle and the initiation of a new cycle. Whether a corpus luteum develops depends on the time in the oestrous cycle at which the operation is performed. If the animal is not in oestrus or if the follicle punctured is small and immature, no changes in the follicle are seen (Day, 1939*a*). In none of the other species in which follicles have been punctured has such a hypertrophy of the theca interna cells as seen in the mare been observed. Yet ovulation in the dog occurs in the early phases of oestrus and such hypertrophy might well be seen if the above explanation of the continued action of the follicular stimulating hormone be correct.

The development of the corpus luteum in the mare is essentially similar to that described by Marshall (1904) for the sheep and Corner (1919) for the sow. The most pronounced differences are the extensive trabeculation to be found in the mare's corpus luteum, and the existence in the early corpora lutea of the lace-like arrangement of the lutealizing granulosa cells, which has been observed also in the fox and the cat.

The comparison of the development of the corpus luteum with the stages of development and the position of the ovum in the genital tract indicate that the corpus luteum has reached its maximum size by the time a morula has been formed and the ovum is nearing the uterus. The corpus luteum would appear to be fully developed at the approximate time of implantation of the ovum (10–14 days). A functional cause for subsequent changes in the corpus luteum cannot yet be given. It is clear from the two series described that the luteal cells develop from the granulosa cells and that the theca interna and theca externa cells invade the luteal tissue from the second day onwards.

SUMMARY

1. An account is given of the development of the Graafian follicle in the mare and the presence of a thecal gland in mature and atretic follicles is demonstrated.

2. The thecal gland is most developed just before ovulation. After ovulation the cells atrophy, lose their vacuolated appearance, and are incorporated in the corpus luteum among the luteal cells.

3. The early development of the corpus luteum is described in a series of twenty-two animals. The luteal cells are derived from the granulosa cells.

4. The corpus luteum reaches its maximum development by the 10th–14th day after ovulation.

5. At this time the luteal cells begin to show a vacuolated outer zone and an inner perinuclear homogeneous area.

6. The histological changes in a series of six artificially ruptured follicles are given.

7. There is a marked hypertrophy of the theca interna cells, similar to the lutealizing changes in granulosa cells, 5–10 hr. after rupture.

8. The granulosa cells do not show lutealizing changes until 18 hr. after rupture.

9. The theca interna cells start to diminish in size and show vacuolation, and invade the luteal cells 21 hr. after rupture.

10. The possible significance of the findings in the ruptured follicles is discussed.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Photograph of a section through the follicle wall on the 1st day of oestrus. The theca interna cells are flattened and placed circumferentially. They fill the area between the two white marks on the photograph. Stained with haematoxylin and eosin. ×650.

Fig. 2. Photograph of a section through the follicle wall on the 4th day of oestrus. The theca interna cells are polyhedral and irregularly placed. They lie between the two white marks on the photograph. Stained with haematoxylin and eosin. ×600.

Fig. 3. Photograph of a section of the developing corpus luteum of the compact type (18 hr.). Stained with haematoxylin and eosin. ×70.

Fig. 4. Photograph of a section of the developing corpus luteum (18 hr.) stained with Robb Smith's modification of Foot's method. The trabeculae show up distinctly. There is little reticulum laid down round the luteal cells. ×90.

Fig. 5. Photograph of a section of the developing corpus luteum (24 hr.) showing the 'lace-like' structure of the developing luteal tissue. Stained with haematoxylin and eosin. ×60.

Fig. 6. Photograph of a section of the developing corpus luteum (4 days) showing vascularization of the trabeculae and invasion of the luteal cells by the theca interna cells. Stained with haematoxylin and eosin. ×70.

Fig. 7. Photograph of a section of the developing corpus luteum (6 days). Stained with haematoxylin and eosin. ×60.

PLATE 2

Fig. 8. Photograph of a section of the fully developed corpus luteum (14 days) showing extensive vacuolation of the luteal tissue. Stained with Masson's stain. ×90.

Fig. 9. Photograph of a section of the fully developed corpus luteum (14 days) stained with Robb Smith's modification of Foot's method. The luteal cells are surrounded by strands of reticulum. ×90.

Fig. 10. Photograph of a section through an artificially ruptured follicle. The animal was killed 2 hr. after the follicle had been punctured. The granulosa cells are intact and unchanged. Stained with Heidenhain's haematoxylin. ×150.

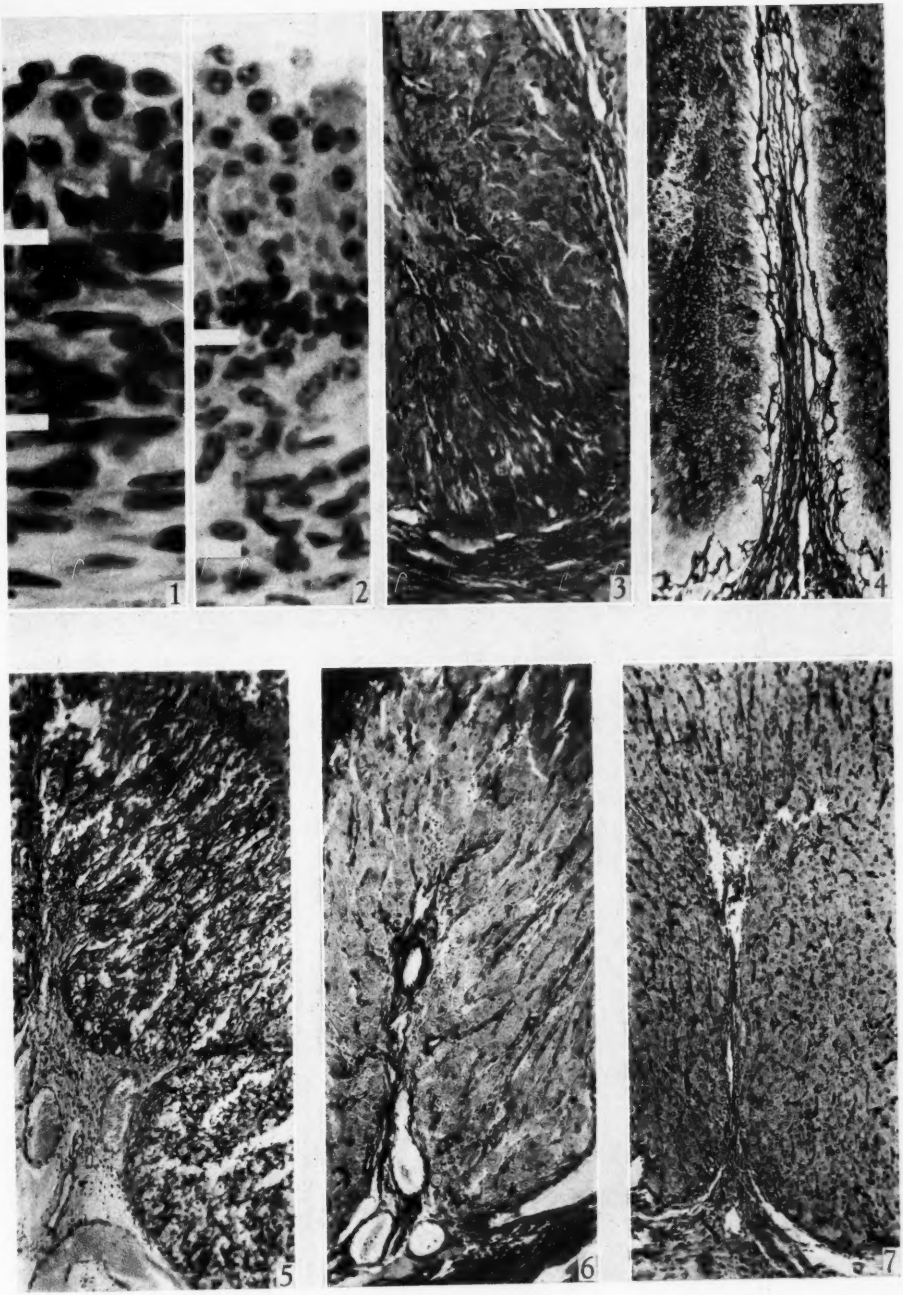
Fig. 11. Photograph of a section through an artificially ruptured follicle. The animal was killed 5 hr. after the follicle had been punctured. The granulosa cells are unchanged, but the theca interna cells show hypertrophy. Stained with haematoxylin and eosin. ×150.

Fig. 12. Photograph of a section through an artificially ruptured follicle. The animal was killed 8 hr. after the follicle had been ruptured. The granulosa cells remain unchanged. Tufts of hypertrophying theca interna cells can be seen projecting into the cavity of the collapsed follicle. Stained with Heidenhain's haematoxylin. ×20.

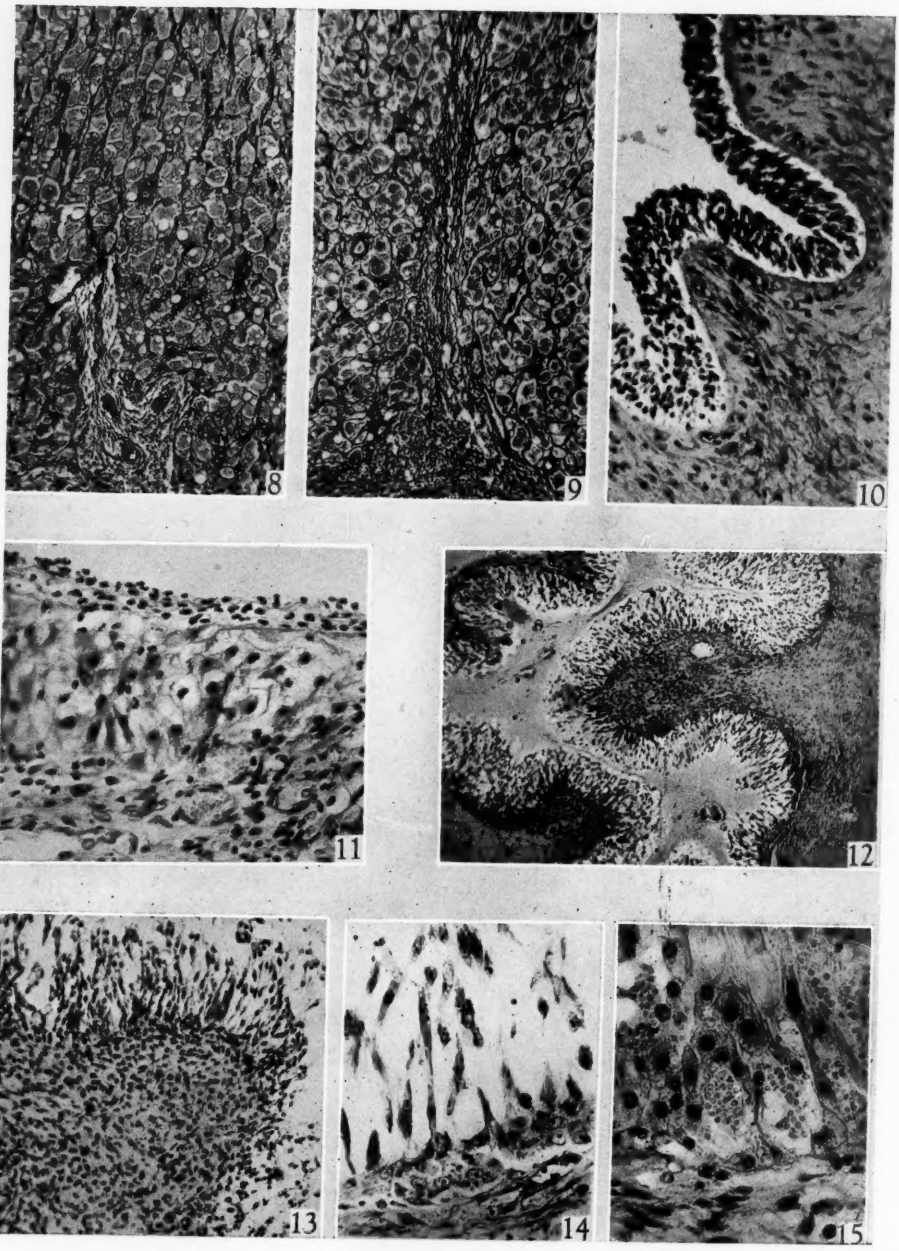
Fig. 13. Photograph of a section through a tuft of theca interna cells projecting into the cavity of an artificially ruptured follicle. The animal was killed 10 hr. after the follicle had been punctured. Stained with Heidenhain's haematoxylin. ×70.

Fig. 14. Photograph of a section through the wall of an artificially ruptured follicle. The animal was killed 10 hr. after the follicle had been ruptured. The granulosa cells are elongated and stretch out into the cavity of the collapsed follicle. The theca interna cells do not show such marked hypertrophy as in the thecal tufts in the previous photograph. Stained with Heidenhain's haematoxylin. ×150.

Fig. 15. Photograph of a section through the wall of an artificially ruptured follicle. The animal was killed 21 hr. after the follicle had been punctured. The granulosa cells show evidence of lutealization. The theca interna cells are much diminished. Stained with haematoxylin and eosin. ×150.



HARRISON—The early development of the corpus luteum in the mare



HARRISON—THE EARLY DEVELOPMENT OF THE CORPUS LUTEUM IN THE MARE

IN MEMORIAM

RAOUL ANTHONY
1874—1941

Many members of the Anatomical Society of Great Britain and Ireland will be sorry to hear of the death, on 1 September 1941, of Prof. Raoul Anthony, of the National Museum of Natural History, Paris.

We are indebted to his old pupil, Dr J. K. Gan, of the Anatomical Laboratory, Toulouse, for the following brief record of Anthony's life and work.

At the age of twenty, while a student at a military college at Lyons, he came under the influence of Testut, who inspired him with the value and importance of comparative anatomy and anthropology.

When he was twenty-nine he was appointed Assistant to Prof. E. Perrier, in the Department of Comparative Anatomy in the Muséum National d'Histoire Naturelle, Paris, and later he became the Sub-Director, finally succeeding his old chief as Professor.

His name is associated with that of Broca whom, in 1911, he was invited to succeed at the École d'Anthropologie, Paris; Manouvrier, whom he succeeded at the École des Hautes Études; Sir Arthur Keith and Prof. E. Loth, with whom he shared the honour of being one of the founders of the Comité International pour l'Anthropologie des parties molles.

Anthony belonged to the old school of anatomists to whom nothing biological was foreign. He was interested in anatomy, embryology, ethnology, psychology, systematic zoology and teratology. He had done research work on nearly every order of mammals, and on invertebrates as well as vertebrates. Furthermore, his studies considered every part of the anatomy of the animal. He was, as his old friend Sir Arthur Keith has described him in a letter, the 'last of the Universalist Anatomists'.

He was a good Greek scholar, and was interested also in mediaeval history.

The following publications indicate the wide field of his biological interests:

Introduction à l'étude experimentale de la morphogénie (1903). *Bull. Soc. Anthrop. Paris*, **4**, 119.

L'encéphale de l'homme fossile de la Chapelle-aux-Saints (with Boule, M.) (1911). *Anthropologie, Paris*, **22**, 129.

Morphology of the shoulder girdle (1913). *17th Int. Congr. Med.*

Les organes de locomotion aérienne chez les vertébrés volants (1913). Paris: Chiron.

Étude expérimentale des facteurs déterminant la morphologie cranienne des mammifères dépourvus de dents. (1913). *C.R. Acad. Sci., Paris*, **159**, 649.

L'encéphale de l'homme fossile de la Quina (1913). *Bull. Soc. Anthrop. Paris*, March.

Ceinture scapulaire chez les batraciens. (with Vallois, H.) (1914). *Bibliogr. anat.* **24**.

Neopallial morphology of fossil men as studied from endocranial casts (with Boule, M.) (1917). *J. Anat., Lond.*, **51**, 95.

La lobation du rein foetal chez les Primates (1923). *C.R. Acad. Sci., Paris*, April.

Le gyrus transversus areae piriformis du cerveau des carnassiers (with Coupin, F.) (1925). *J. Anat., Lond.*, **59**, 113.

L'indice de valeur cérébrale (with Coupin, F.) (1925). *Rev. anthrop.* **35**, 145.

Developpement du cerveau de l'ours brun (*Ursus arctos* L.) (1926) *J. Anat., Lond.*, **60**, 449.

Les affinités de cétacés (1926). *Ann. Inst. Océanogr. Monaco.*

Anatomie comparée du cerveau (1928). Paris: O. Doin.

Le Neopallium des Équidés (with Grzybowski, J. de) (1930). *J. Anat., Lond.*, **64**, 147.

JOHN ERNEST SULLIVAN FRAZER, D.Sc., F.R.C.S.

Emeritus Professor of Anatomy in the University of London

1870–1946

The passing of Prof. J. E. S. Frazer removes one more of the great figures of the older generation of anatomists. Unfortunately, he had been unable for the past nine years to take the same active part as before in anatomical proceedings, owing to the serious illness which seized him suddenly while in the full vigour of his activities. Since that time he had had to restrict himself to less onerous work, such as the revision of his publications, but he retained throughout his interest in the subject which had been his life's occupation.

Frazer was born in London in 1870, and was educated at Dulwich College. In his early years he had considered following the profession of art, for which he showed some inclination and aptitude. The project was discarded in favour of his other leaning, which was towards medicine, and on leaving school he entered St Bartholomew's Hospital, from which he qualified at the early age of 21. Valuable practical experience was gained by a number of medical and surgical appointments during the period which followed. In particular, he was for some years at the Ventnor Hospital, where he learned much about the medicine and surgery of the chest. He took the F.R.C.S. in 1895, intending to become a surgeon, but a severe post-mortem wound, which for a time threatened his life, caused him to give up this idea, and to make the study of anatomy, which had attracted him always, the primary focus of his career, instead of a subsidiary subject. He obtained a demonstratorship at St George's Hospital, and in 1905 went to King's College, where he worked with Peter Thompson. In 1911 the Medical School at St Mary's was undergoing a change which, in the event, was the beginning of his long association with that Hospital. The authorities decided to fall in line with the other schools, and to appoint a full-time lecturer in Anatomy. Up to that time the surgeons had the responsibility of providing lectures and demonstrations, and there was no full-time teacher in the department. Frazer was appointed to the post, and became the first full-time lecturer in Anatomy.

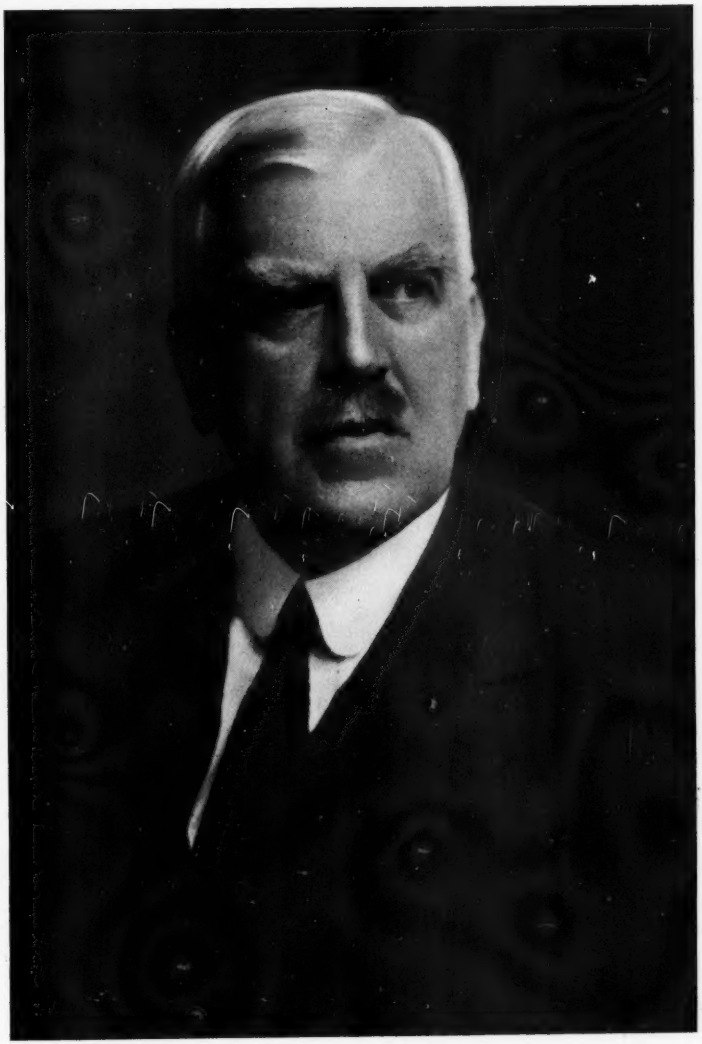
It was uphill work, owing to the meagre accommodation and facilities available in the dilapidated old building which constituted at that time the Medical School. His room consisted of a tiny partitioned-off portion of the dissecting room, and contained neither water nor gas, both of which had to be obtained in other parts of the main room. In 1914 he was appointed Professor in the University of London, and a room was then found for him on the floor below. But it was not until 1932 that suitable accommodation was provided by the erection of the present building. In spite of these difficulties he was able at once to give proof of his great ability both in teaching and in research.

As a teacher he compelled the interest of his hearers by his enthusiasm and by his direct informal style. His gift of pictorial illustration assisted considerably. He would build up a region on the blackboard, superimposing the structures step by step. In addition he used full-sized coloured drawings, in which, owing to his skill in light and shade, the details were clearly visible even in the back rows, a reminder of his early artistic bent. Topographical anatomy, although not holding first place, had a great fascination for him. At one time he even contemplated writing a book on relations, and actually made a start, but came to the conclusion that the difficulty of keeping its scope within reasonable limits made it impracticable. He was wont to insist that what appeared to be a minor detail might at any time constitute a determining factor in the diagnosis of an obscure case, and would give personal experiences in support of his contention.

But it was in the field of embryology that he showed his great powers of careful and thorough investigation. He was constantly preparing and examining specimens of different ages, since, as far as possible, he liked to base his opinions on personal observation. As in topographical anatomy also, his golden rule for clearing up any doubtful point was that one should get hold of the necessary material and see for oneself.

But he also paid due attention to the work of others, assessing it according to the degree to which, after reading the original papers, he considered they proved their case. His memory was extraordinary; he would give the gist of a contribution he had read ten years or more previously, naming the journal, the author, and the approximate date. One felt that any judgement he formed was with full knowledge of all important contributions to the subject.

His own researches included portions of all the systems of the growing embryo, his work on the



JOHN ERNEST SULLIVAN FRAZER

1870–1946

UNIV. OF MICH.

pharynx, larynx, and tubo-tympanic region being especially notable. The period covered by this investigation was 1910–14, when papers were published on 'The development of the larynx' (1910), 'Development of the nasal cartilages' (1911), and 'The second visceral arch and groove in the tubo-tympanic region' (1914). A further paper on the 'Early formation of the middle ear and Eustachian tube' appeared in 1923.

The septation of the heart engaged his attention at another time, particularly that of the difficult ventriculo-bulbar region. He formulated his conclusions in 'The formation of the pars membranacea septi' (1917). Another region which attracted him on account of its obscurity was the mid-brain, on which he made some very intriguing observations in 'The development of the region of the isthmus rhombencephali' (1929).

Other contributions to embryology were: 'The derivation of the hypothenar muscles' (1908); 'The formation of the duodenal curve' (1919); 'The disappearance of the pre-cervical sinus' (1927), and, with a collaborator, 'Factors concerned in the rotation of the intestine' (1916) and 'The development of the lower end of the vagina' (1928).

In addition he produced several books. His *Anatomy of the Human Skeleton* showed his wizardry in making the dry bones live. It has reached a fourth edition and has travelled to all parts of the world. His *Manual of Embryology* is the record of his own researches over a period of 25 years. It comprises not only the work that is to be found in his numerous papers and in well-known text-books, but a good deal also that makes its appearance for the first time. Ordinary descriptions are followed except where his own observations point to a different conclusion. In this way the book represents a personal estimate and review of the present state of knowledge of human development. He rewrote Buchanan's anatomy, one of the few books on anatomy that are for reading rather than reference. It is an excellent text-book, and the embryology of the structures is dealt with simply and in the appropriate place. Finally he produced, with myself, a *Manual of Practical Anatomy*.

He became a member of the Anatomical Society in 1902, and was successively a member of the Council, Secretary (1915–19), and President (1935–7). He was Hunterian Professor in the College of Surgeons (1915–16), Harveian lecturer (1924), Chairman of the Board of Studies in anatomy and morphology in the University of London, examiner in the universities of London, Durham, Oxford and Cambridge, and Secretary of the section of Anatomy and Physiology at the annual meeting of the British Medical Association at Birmingham (1911). During the first world war he acted as Outpatient Surgeon.

He received the D.Sc. London in 1932, and following his retirement in 1940 was made Emeritus Professor.

He was of fine physique, and as a young man was a prominent member of the United Hospitals Athletic Club, winning several cups and medals. He excelled especially in throwing the hammer.

As a man, his geniality, sense of humour, and readiness to listen as well as talk, made him a delightful companion. His advice was sound, but was not insisted upon. His upright figure and generous proportions seemed in harmony with his inner nature. His friendship was real, practical and enduring.

He leaves a widow and one son, and to them we extend our sincere sympathy. R. H. R.

Just before going to Press we learn with great regret of the death on 16 May of Emeritus Professor Thomas Hastie Bryce, F.R.S. An Obituary Notice will appear in the next number.